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ERRATA AND AUTHORS' EMENDATIONS

- Page 48, third line from bottom, "distilled" should be "each mixed."
- Page 99, figure 2, *B*, "Ward City" should be "Ward Creek."
- Page 100, sixth line, "Humboldt Range" should be "Humboldt Basin."
- Page 120, seventeenth line, "horizontal" should be "elevation."
- Pages 125 and 126, the legend of figure 9 belongs to figure 10 and the legend of figure 10 belongs to figure 9.
- Page 235, footnote 1, "University of Kansas" should be "University of Arkansas."
- Page 252, nineteenth line, "vitamin (I)" should be "vitamin B."
- Page 318, reference 2, "Holz-spund" should be "Holz-und", and reference 12, "Azetyl-spund" should be "Azetyl-und."
- Page 344, legend for figure 2, "*irregulare*" should be inserted after "*sigmoideum*."
- Pages 388-396, "whitefish meal" wherever used should be "white fishmeal."
- Page 389, lines 33-35, figures should align, ⁸⁹
81, 108, and 79
⁹⁶ ⁸⁸
- Page 390, second heading at bottom of page should be "50 percent protein grade" instead of "55 percent protein grade."
- Page 396, third line, "haddock meals" should be "white fishmeals."
- Page 396, seventh line from bottom, "solvent" should be "expeller."
- Page 402, first line under Experimental Procedure should read "Corn-gluten meal, linseed meal, and soy-bean-oil meal were each substl."
- Page 430, table 1, third line, last column, "3.700" should be "3.300."
- Page 430, table 1, next to bottom line under heading "basal area by-", "7.4128, 7.398, 7.392, and 7.412" should be "0.4128, 0.398, 0.392, and 0.412", respectively
- Page 454, legend for plate 2, all magnifications should be reduced 25 percent.
- Page 522, first line, "Elsinoë" should be "Elsinoë."
- Pages 721 and 724 Figure 1 should be figure 3 and figure 3 should be figure 1.
- Page 739, ninth line from bottom, "about 2 to 1,000 dilution" should be "about 1-2000 dilution."
- Page 784, twenty-eighth line, delete the words "exterior surfaces of the."
- Page 794, sixth line from bottom, "plates S" should be "plate 8."
- Page 833, table 3, second line, omit "Petals narrow, margins crimped—Continued"
- Page 864, legend for figure 7, *B*, magnification should be "403" instead of "203"
- Page 876, figure 15, *A*, "*ti*" should be "*ti*"
- Page 925, second line from bottom, "an apple" should be "on apple"
- Page 1002, legend for figure 12, *B*, should read "section from a primary lateral illustrating the origin of a very small branch, shown about natural size."

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No. 1

THE EFFECTS, ON CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS, OF FEEDING LOW-CALCIUM RATIONS FOR LONG PERIODS¹

By EDWARD B. MEIGS, *senior physiologist*, WILLIAM A. TURNER, *associate chemist*, EDWARD A. KANE, *assistant chemist*, and LEO A. SHINN, *junior chemist*, *Division of Dairy Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture*²

INTRODUCTION

A study of the numerous experiments in which calcium and phosphorus balances of dairy cows have been determined during the last 25 years gives a confusing impression. Some of the experiments indicate that cows readily lose these elements from their bodies, and are likely to lose them at a rapid rate if they are giving any considerable quantity of milk. Thus, Hart, McCollum, and Humphrey (6)³ obtained results in 1909 which would indicate that a cow giving from 12 to 18 kg of milk daily and fed on rations low in calcium lost about 2 kg of calcium from her body in the course of 111 days, or more than 25 percent of all the calcium her body might have been expected to contain. Since then the calcium and phosphorus metabolism of dairy cows has been studied in many experiments at several American experiment stations. The results of the earlier experiments at Beltsville, Md. (15), and of later ones at the Wisconsin (7) and Beltsville (28) Stations indicate (1) that milking cows are likely to be losing calcium from their bodies even when their rations contain large amounts of calcium, (2) that on rations low in calcium, the losses are likely to be larger, and (3) that it is probably difficult to keep cows in calcium equilibrium when they are subjected to the usual dairy regime. The practical conclusion from such results is, of course, that it is wise to feed liberally milking cows on rations that contain large amounts of calcium.

On the other hand, certain recent work at the Michigan (12) and Vermont (4) Stations indicates that mature cows which are fed for several years on rations low in calcium may readily assimilate enough calcium from such rations to supply what is needed for their milk, and may sometimes even be storing considerable amounts of it in their bodies. Some results of the Vermont work indicate also that calcium and phosphorus may be stored in proportions very different from those in which they are usually found in mammalian bone or in the mammalian body.

¹ Received for publication Mar. 7, 1935; issued August 1935.

² The writers acknowledge the help given by P. E. Howe, O. G. Hankins, N. R. Ellis, and other members of the Bureau of Animal Industry, U. S. Department of Agriculture. The work would have been difficult or impossible without the advice and help received from them on all the details of the slaughter analyses.

³ Reference is made by number (italics) to Literature Cited, p. 24.

In carrying out balance experiments which extend over long periods it is not easy to avoid small errors in sampling and making analyses which might accumulate in the course of the experiment and appear in the final results as large distortions of the total quantities of calcium and phosphorus assimilated. Some of the Vermont results suggest strongly to anyone familiar with the usual proportions of calcium and phosphorus in body and bone that such errors have occurred. It seems possible also that such errors may partly explain the surprisingly large losses of calcium and phosphorus that appear to have occurred in other balance experiments.

It does not seem justifiable, however, to discard on mere suspicion the results of the great amount of work represented by balance experiments. Fortunately, analyses of the whole bodies of cattle provide a satisfactory though laborious method of checking many aspects of the balance experiments. There already exists a large amount of information which throws much light on the limits of variability in the normal calcium and phosphorus content of the bovine body and bone.

In the present study the results of previous body analyses of cattle have been used in conjunction with the results of new experiments to make a beginning toward showing how far the normal quantities of calcium and phosphorus in body and bone are likely to be altered by subjecting the animals to long periods on rations low in calcium.

OUTLINE OF STUDY

The experimental work to be described and discussed may be divided into three groups.

In the first group the calcium intake in the feed and the calcium output in the milk and calves of cows was recorded over long periods, at the end of which the animals either died naturally or were slaughtered. Analyses were then made of several of the bones of the cows, and a rough attempt was made to estimate whether the feeding of rations low in calcium had any tendency to reduce the weight of the bones in relation to that of the rest of the body.

But, as this method of estimating changes in the calcium and phosphorus content of the body is unsatisfactory for many reasons, a second group of experiments was carried out in which the intake of calcium in the feed and the output through the milk and calves of three cows over periods of several years was determined. Two of these cows were on rations containing liberal proportions of alfalfa hay and the third was on a ration of grain with timothy hay as the sole roughage. At the end of the experimental period the three cows were slaughtered, and determinations were made of the calcium, phosphorus, fat, nitrogen, ash, and water in their entire bodies.

A third group of experiments consisted in determining the calcium and phosphorus balances of cows which had been on low-calcium rations for over a year, and were still on low-calcium rations at the time the balances were determined.

PREVIOUS BODY ANALYSES OF CATTLE

Determinations of fat, calcium, phosphorus, and other mineral elements in the bodies of cattle were carried out many years ago by Lawes and Gilbert of Great Britain (14), and much more recently by

Trowbridge, Moulton, Haigh, Hogan, and others at the Missouri Agricultural Experiment Station (5, 11, 16, 17, 18, 19, 23). In the Missouri investigation the animals were fed in various ways, though not in ways that would be expected to produce any very marked changes in the calcium and phosphorus content of the body. A study of the results, however, throws much light on the relationship between the calcium and phosphorus content of the bodies of cattle, and on the variations which are to be expected in the calcium, phosphorus, and fat content at different ages and under different conditions of feeding.

The American investigations were much more extensive than the British ones and will be considered first. Hogan and Niernan (11) give figures for analyses of a few embryos, a number of calves slaughtered at various ages from just after birth to 8 months old, and a number of steers about 4 years old. The animals were fed on rations which were generally similar in make-up to rations fed under good conditions of farm practice, and all contained fairly liberal proportions of calcium. Three planes of nutrition were used, however, for the study; the first, high enough to permit marked fattening; the second, sufficient to permit good growth without much fattening; and the third, so low that growth as well as fattening was considerably restricted. The results show that while the body fat varies greatly according to the plane of nutrition, the percentages of calcium and phosphorus in the fat-free bodies of the animals are little affected. This point is brought out by the figures in table 1. The thin animals have only about one-third as much fat in their bodies as the fat ones, but about 1.6 times as much calcium and phosphorus. The calcium and phosphorus content of the fat-free bodies, on the other hand, varies comparatively little, there being less than 10 percent difference between the highest and the lowest figures for either element.

TABLE 1.—Average calcium, phosphorus, and fat in the whole bodies and in the fat-free bodies of steers from 40 to 48 months old fed at different planes of nutrition¹

IN WHOLE BODIES			
Plane of nutrition	Calcium	Phosphorus	Fat
	Percent	Percent	Percent
High.....	0.98	0.53	44.21
Medium.....	1.51	.80	20.43
Low.....	1.59	.84	14.06
IN FAT-FREE BODIES			
High.....	1.75	0.94	-----
Medium.....	1.90	1.01	-----
Low.....	1.86	.99	-----

¹ The figures for calcium and phosphorus are from Missouri Research Bulletin 107 (11); those for fat are from Missouri Research Bulletin 55 (18).

The manner in which the calcium and phosphorus content changes with advancing age in the fat-free bodies and in the fat-free soft tissues of cattle, as brought out by calculations from the Missouri data, is shown in table 2.

TABLE 2.—*Calcium and phosphorus in fat-free bodies and fat-free soft tissues of cattle at various ages, as calculated from Missouri data*FAT-FREE BODIES¹

Kind, and age of animal	Calcium	Phosphorus
	Percent	Percent
Fetus, 232 days old.....	0.879	0.493
New-born calves (average of 5).....	1.241	.708
Steers (average of 9, 3 to 8 months old).....	1.453	.840
Steers (average of 9, 40 to 48 months old).....	1.838	.978

FAT-FREE SOFT TISSUES²

Steers (average of 3):		
3 months old.....	0.022	0.186
5 months old.....	.022	.170
8 months old.....	.021	.159
40 months old.....	.016	.140
44 months old.....	.016	.144
48 months old.....	.017	.143

¹ Calculated from Missouri data (11).² Calculated from Missouri data (11, 18).

Table 2 shows that the percentage of calcium and of phosphorus in the fat-free bodies of cattle tends to increase materially up to the period of maturity. Experiments like those which are to be described in this paper should, therefore, be confined to mature cattle. Table 2 also shows that the percentage of calcium and of phosphorus in the fat-free soft tissues of cattle is small and fairly constant, but that both the calcium and phosphorus tend to decrease with advancing age.

Still other points of interest brought out by the figures in table 2 are that the calcium content of the soft tissues of cattle is very small; that the phosphorus content is larger, but still only a rather small fraction of that contained in the bones; and that the ratio between the calcium and phosphorus contained in the whole body tends to be fairly constant. The calcium contained in the soft tissues of mature steers is less than 1 percent of that contained in the whole body, while the phosphorus in the soft tissues is about 15 percent of the total. The approach to constancy of the Ca/P ratio in the whole body is dependent on the still more nearly constant Ca/P ratio in bone. In the whole series of animals whose bone analyses are given by Hogan and Nierman (11), the Ca/P ratio did not fall below 2:1 or rise above 2.14:1 for the skeleton of any single animal. These figures are a little lower and a little less constant than they might be because of the fact that the skeletons were only roughly cleaned. In the carefully cleaned bones used in the investigation by Hartman and Meigs (8), the Ca/P ratio did not rise above 2.23:1 or fall below 2.14:1 for any single bone.

The effects of advancing age and of rations containing different amounts of calcium and phosphorus, on the relative amounts of various mineral elements in bones, have been studied by Neal, Palmer, Eckles, and Gullickson (20), and by Henderson and Weakley (9). The former group of investigators found that the ratio between calcium phosphate and calcium carbonate in bone decreases with advancing age and is also reduced by feeding rations low in phosphorus. The changes, though definite, are not very large, and would not produce any very great change in the ratio between the total calcium and the total phosphorus of the bone.

Henderson and Weakley (9) give figures for the total calcium and total phosphorus of the bones of cattle at various ages, that had been fed rations containing various amounts of calcium and phosphorus. Their results are in entire general agreement with those of Neal and his collaborators in showing that there is slightly more calcium in relation to phosphorus in the bones of older cattle and in those of cattle fed on rations low in phosphorus, but the differences are very small. Table 3 gives figures for the Ca/P ratios calculated from the data of Henderson and Weakley. These ratios do not fall below 2.06:1 nor rise above 2.20:1 in any case. The Ca/P ratio is obviously more affected by changes in age than by changes in feeding.

TABLE 3.—*The calcium-phosphorus ratio in the bones of cattle at various ages, that had been fed on rations containing various amounts of calcium and phosphorus, as calculated from the data of Henderson and Weakley (9)*

Character of ration	Ca/P ratio in bones of cattle, at—				Average
	8 months	13 months	19 months	25 months	
Normal	2.09	2.12	2.10	2.15	2.11
Low in calcium	2.06	2.10	2.11	2.14	2.10
Low in phosphorus	2.10	2.13	2.19	2.17	2.15
Low in both calcium and phosphorus	2.08	2.10	2.17	2.20	2.14
Average	2.08	2.11	2.14	2.16	

The studies made by Lawes and Gilbert (14) on cattle were much less extensive than those at the Missouri Station. As far as they go, however, they agree very closely with the Missouri results. Table 4 shows how close this agreement is for the calcium and phosphorus content of the fat-free bodies of cattle at different ages.

Lawes and Gilbert give no figures for the composition of the separated skeletons and soft tissues of their animals, but, by assuming that the soft tissues of their animals had the usual calcium content and that the bones had the usual proportional weight and Ca/P ratio, the phosphorus of the soft tissues can be estimated. The results of such calculations indicate that their animals had very nearly the same percentages of phosphorus in their fat-free soft tissues as the corresponding animals of the Missouri investigation.

TABLE 4.—*Average calcium, phosphorus, and fat in whole bodies and in fat-free bodies of cattle at about 3 months of age and at about 4 years of age, as indicated by 2 investigations*

Investigation	Calves, at about 3 months of age					Oxen, at about 4 years of age				
	Whole bodies			Fat-free bodies		Whole bodies			Fat-free bodies	
	Calcium	Phosphorus	Fat	Calcium	Phosphorus	Calcium	Phosphorus	Fat	Calcium	Phosphorus
Lawes and Gilbert (14)	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Missouri	1.18	0.67	14.80	1.38	0.79	1.39	0.74	24.60	1.84	0.98
	1.34	.78	7.15	1.44	.84	1.36	.72	26.60	1.85	.98

There is a very satisfactory agreement, therefore, between the older British and the newer American investigations. From a study of the figures one gets the impression that the methods of determining calcium and phosphorus in the tissues of cattle were quite adequate as far back as 1859, and that Lawes and Gilbert were able to determine these elements with just about the same degree of accuracy as can be attained at the present time. One gets the impression also that the calcium and phosphorus content of the tissues of cattle varies only within narrow limits and according to certain definite laws.

This impression is strengthened by the fact that results very similar to those just discussed have been obtained in numerous investigations of the composition of the whole bodies of other species of animals, and of particular tissues of cattle as well as of other animals. It is not necessary to describe these at length, but it may be mentioned that Sherman (24, 25) has shown that the proportional amounts of calcium and phosphorus in the bodies of rats increase with advancing age and that Katz (13) has obtained figures for the phosphorus content of ox and calf muscle which are very similar to those found in the Missouri investigations. Katz found 0.170 percent of phosphorus in ox muscle and 0.220 percent in calf muscle.

EXPERIMENTS IN GROUP 1

PRELIMINARY ESTIMATES OF CALCIUM ASSIMILATION IN LONG-CONTINUED FEEDING EXPERIMENTS FOLLOWED BY SLAUGHTER AND BONE ANALYSIS

The work reported in this section is a continuation and enlargement of work already published (8). The procedure employed has been fully described (8), and need only be outlined here. In the tabulated results the breed of all cows at the Beltsville Station is indicated by their numbers. Those numbered from 1 to 99 and from 300 to 399 are grade Jerseys; those numbered from 100 to 199, grade Holsteins; those numbered from 200 to 299, registered Holstein-Friesians; and those numbered from 400 to 499, registered Jerseys. The grain mixtures used are described in table 5.

TABLE 5.—Composition of grain mixtures ¹ used in the group 1 experiments

Grain ration no.	Corn meal	Wheat bran	Cotton-seed meal	Soybean meal	Linseed meal	Salt
	<i>Parts</i>	<i>Parts</i>	<i>Parts</i>	<i>Parts</i>	<i>Parts</i>	<i>Parts</i>
50.....	40	30	20	-----	10	1
55.....	30	20	25	-----	25	1
60.....	40	30	-----	20	10	1
65.....	30	20	-----	25	25	1
75.....	40	40	-----	-----	20	1

¹ The corn meal used throughout the experiments was yellow. The oil meals had their fat extracted by the old process, that is, without fat solvents.

The calcium intake in the food of cows and the output through their milk and calves was determined for periods of from 1 to 6 years. At the end of these periods the cows either died or were slaughtered, and some of their bones were carefully cleaned, weighed, and analyzed. Some of the cows received liberal amounts of alfalfa hay in the experimental periods; others received only timothy hay or timothy hay and corn silage as roughage. From the figures for the calcium intake and output determined as above described, the percentage of food calcium utilized for the production of milk and calves can be calculated; the result has been called the "percentage calcium assimilation." This result, however, does not represent the true percentage of food calcium utilized, unless it can be assumed that calcium is neither gained nor lost by the body during the experimental period. In order to obtain an idea of how much calcium is likely to be lost by the body of a cow in a period of 2 or 3 years on timothy hay, studies of the bones of cows have been made as above outlined after periods on timothy and on alfalfa, respectively. These studies suggest that the losses on timothy are not likely to be large enough seriously to vitiate the determinations of percentage calcium assimilation in periods lasting more than 2 years, but the results obtained in the experiments under consideration are only approximate and leave many questions to be answered by the later complete slaughter analyses.

Tables 6 to 9 give a summary of the results already published, together with the results of new experiments to be described later.

TABLE 6.—*Ratio of bone weight to body weight in cows when fed alfalfa hay or timothy hay*

ALFALFA HAY

Cow no.	Ratio of bone weight to body weight in—	
	Long bones	Ribs
17	0.00545	0.00191
8400555	.00186
9000576	.00188
N20200621	.00176
45800540	.00185
Average00569	.00181

TIMOTHY HAY

N401	0.00589	0.00155
42900491	.00155
49900525	.00155
Average00535	.00155

TABLE 7.—Total amounts of feed and of calcium from the various feeds consumed by the cows when fed rations containing or lacking alfalfa hay

RATIONS CONTAINING ALFALFA HAY

Cow no.	Experimental feeding period	Feed and calcium consumed							
		Grain		Alfalfa		Timothy		Silage	
		Total	Calcium	Total	Calcium	Total	Calcium	Total	Calcium
		Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams
84.....	Jan. 1, 1927, to Oct. 28, 1932	¹ 10, 768	12.6	9, 158	124.9	1, 616	5.1	8, 225	8.2
N202 ²	Jan. 1, 1927, to Aug. 20, 1929	³ 5, 113	6.6	4, 892	62.2	-----	-----	-----	-----
458.....	Jan. 1, 1927, to Jan. 13, 1933	⁴ 12, 052	15.4	11, 057	152.7	1, 846	5.8	-----	-----

RATIONS CONTAINING NO LEGUME HAY

Cow no.	Experimental feeding period	Total	Calcium	Total	Calcium	Total	Calcium	Total	Calcium
		Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams	Kilo-grams
54.....	Nov. 1, 1924, to Jan. 31, 1927	⁵ 4, 899	7.9	-----	-----	2, 852	8.2	5, 335	5.2
N401.....	Apr. 1, 1929, to Dec. 24, 1931	⁶ 0, 457	13.3	-----	-----	4, 651	17.9	-----	-----
429.....	Dec. 1, 1924, to May 31, 1927	⁷ 5, 828	9.3	-----	-----	3, 283	9.6	5, 819	5.7
450.....	Oct. 1, 1925, to Nov. 30, 1926	⁸ 2, 011	3.2	-----	-----	1, 678	4.8	-----	-----
456.....	Aug. 1, 1924, to Mar. 31, 1927	⁹ 4, 767	7.6	-----	-----	3, 240	9.2	4, 972	4.9
499.....	Oct. 1, 1927, to Dec. 16, 1932	¹⁰ 12, 851	24.8	-----	-----	7, 532	22.9	-----	-----

¹ 6,595 kg of grain 50, and 4,163 kg of grain 60.² In addition to grain and hay, cow N202 consumed 3,576 kg of beets and 377 kg of beet pulp in the experimental period, containing 3 kg of calcium.³ Grain 60.⁴ 11,917 kg of grain 60, and 135 kg of grain 75.⁵ Grain 55.⁶ Grain 65.

TABLE 8.—Intake and outgo of calcium and percentage of calcium assimilated by cows fed various rations

Cow no.	Ration	Length of period	Total calcium in—			Calcium assimilated ¹	Total milk yield
			Food	Milk	Calves		
		Days	Kilograms	Kilograms	Kilograms	Percent	Kilograms
84.....	Grain, alfalfa, timothy, silage	2, 128	150.8	17.47	1.67	12.7	13, 929
N202.....	Grain, alfalfa, beets, beet pulp	963	68.8	7.69	1.33	13.1	6, 866
458.....	Grain, alfalfa, timothy	2, 205	173.9	28.79	1.39	17.4	18, 599
54.....	Grain, timothy, silage	822	21.3	5.85	.36	29.2	5, 851
N401.....	Grain, timothy	998	31.2	11.99	.85	41.2	8, 046
429.....	Grain, timothy, silage	912	24.6	10.82	.61	46.5	8, 280
450.....	Grain, timothy	427	8.0	2.42	.21	32.9	1, 731
456.....	Grain, timothy, silage	973	21.7	6.45	.61	32.5	4, 610
499.....	Grain, timothy	1, 904	47.7	21.27	1.43	47.6	14, 598

¹ Percentage of calcium assimilated = $\frac{\text{Ca in milk and calves}}{\text{Ca in food}} \times 100$.

TABLE 9.—Composition of fresh clean bones of cows that had been fed rations containing alfalfa hay and rations containing no legume hay, respectively

GRAIN AND ALFALFA HAY

Cow no.	Ash		Calcium		Phosphorus		Protein ¹		Water		Ca/P ratio	
	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs	Long bones	Ribs
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent		
17.....	48.6	51.4	18.8	19.8	8.7	9.1	20.3	24.9	18.5	17.7	2.16	2.18
90.....	46.8	47.1	18.1	18.2	8.4	8.4	20.6	23.7	11.7	16.0	2.15	2.17
N202.....	47.4	51.0	18.8	19.4	8.7	9.2	20.4	24.4	12.3	14.7	2.16	2.11
Average.....	47.6	49.8	18.6	19.1	8.6	8.9	20.4	24.3	14.2	16.1	2.16	2.15

GRAIN AND TIMOTHY HAY

N401.....	46.5	43.1	17.8	16.4	8.5	7.8	20.7	23.6	12.6	22.0	2.09	2.10
429.....	47.9	46.2	18.7	18.2	8.0	8.2	22.0	25.2	12.2	18.7	2.17	2.22
Average.....	47.2	44.6	18.2	17.3	8.5	8.0	21.3	24.4	12.4	20.3	2.14	2.16

¹ N X 6.25.

The bones which were weighed and analyzed in the experiments under consideration were the radius and ulna, metacarpus, tibia, metatarsus, and the tenth, eleventh, twelfth, and thirteenth ribs from one side of the body. The ratios of bone weight to body weight in table 6 were obtained by dividing the sum of the weights of the long bones and the sum of the weights of the ribs respectively by the body weights of the cows. The figures for body weight were obtained as in the original investigation by averaging the monthly weights of the cows for periods of 2 to 3 years before their deaths, omitting the weights for 4 months before and 2 months after calving.

The figures in table 6 suggest that the loss of material from the long bones of cows fed for long periods on timothy hay is very small. The loss from the ribs appears to be larger, but the results are too few to justify any positive conclusions. For this reason, the figures for calcium assimilation in table 8 have been calculated without making any allowance for possible loss of bone material during the experimental periods. This matter will be discussed after the results of the slaughter experiments have been given. The figures, as they stand, suggest that cows on timothy rations are likely to assimilate much larger proportions of the calcium intake than those on alfalfa rations.

Table 9 gives the composition of the carefully cleaned bones of several cows which had been fed alfalfa and timothy rations, respectively, for long periods. The results show the strong tendency toward constancy in bone composition, and particularly the constancy of the Ca/P ratio. The results also suggest that the ribs are affected more than the long bones by rations low in calcium. The figures in table 9 show that the percentages of ash, calcium, and phosphorus were only slightly lower in the long bones of the cows fed timothy than in those of the cows fed alfalfa, whereas these percentages in the ribs of the cows fed timothy were considerably lower.

While these somewhat fragmentary studies of the bones of cattle that have been fed different rations for long periods suggest that long

periods on rations low in calcium have only a rather small effect on the weight and composition of the bones, such studies are obviously much less satisfactory than would be the determination of the total quantities of calcium, phosphorus, fat, and other materials in the whole bodies of cattle under such circumstances. The results of such determinations are given in the section following.

EXPERIMENTS IN GROUP 2

BELTSVILLE SLAUGHTER EXPERIMENT

The three cows studied were nos. 84, 458, and 499. These cows were fed fairly uniform rations without pasture for approximately 6 years before they were slaughtered. The percentage of calcium assimilated during the experimental periods was determined according to methods previously outlined (8). The results along with those of other cows subjected to somewhat similar treatment are given in table 8. Only a few details need be added here.

Cow 84, born October 11, 1917, was started on the experiment January 1, 1927, on a moderately high-calcium ration, receiving grain 50, alfalfa hay, and corn silage until April 1931. From then to August 1931, she received several different rations containing grain 50, grain 60, alfalfa hay, timothy hay, and corn silage. From August 1931 to the date of slaughter, she received grain 60, alfalfa hay, and timothy hay. Her average daily calcium intake was 70.9 g. Her last calf was born October 15, 1932; she was slaughtered October 28, 1932, and weighed 399.6 kg just before slaughter.

Cow 458, born June 20, 1921, was started on the experiment January 1, 1927, on a high-calcium ration, receiving grain 60 and alfalfa hay through May 1931. From then until September 1932 she received grain 60 with alfalfa and timothy; and from then until the date of slaughter she received grain 60 with alfalfa hay alone. Her average daily calcium intake was 78.9 g. Her last calf was born December 6, 1932; she was slaughtered January 13, 1933, and weighed 394.1 kg just before slaughter.

Cow 499, born April 29, 1923, was started on the experiment October 1, 1927, on a low-calcium ration, receiving grain 65 and timothy hay until the date of slaughter. Her average daily calcium intake was 25.1 g. Her last calf was born November 19, 1932; she was slaughtered December 16, 1932, and weighed 432.6 kg just before slaughter.

All hay fed to these three cows was U. S. No. 1 grade (21). The calcium content of the grain and silage fed was calculated by multiplying the total consumption by average figures for the calcium content of grain and silage, obtained from a number of analyses of samples of these feeds used at the Beltsville Station. Each batch of hay bought was analyzed, and the calcium content of the amount of this batch consumed by each cow was calculated from this analysis. It was thought better to follow this more laborious procedure with the hay on account of the greater variability in its composition.

The milk of each of the three cows was analyzed a number of times, and the averages of these analyses for each cow were used in calculating the calcium content of her milk. Table 10 gives some of the details regarding these analyses, and also a comparison of some of the Beltsville figures for the feeds with the average figures given by Henry and Morrison (10).

TABLE 10.—*Data regarding the calcium analyses of the feed and milk of cows 84, 458, and 499 in the experimental periods previous to slaughter*

Material analyzed	Analyses	Calcium found in Beltsville analyses			Average calcium figures of Henry and Morrison ¹
		Maximum	Minimum	Average	
	<i>Number</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Alfalfa hay	27	2.406	0.962	1.424	1.393
Timothy hay	20	.564	.175	.322	.179
Grain 50	5	.129	.093	.111	.112
Grain 60	35	.157	.102	.128	-----
Grain 65	18	.233	.171	.193	-----
Corn silage	4	.104	.093	.100	-----
Milk from—					
Cow 84	5	.131	.114	.125	-----
Cow 458	5	.158	.148	.155	-----
Cow 499	14	.157	.134	.146	-----

¹ Henry and Morrison (10) give no figures for soybean meal from which the fat has been extracted, or for corn silage.

The animals were slaughtered in the usual manner by a blow on the head followed by bleeding. The blood was collected in a tub and weighed. To determine its composition samples were obtained shortly before slaughter and analyzed.

After the animals had been drained of blood, the hides were removed and weighed. Representative samples were then cut from them as described by Small (26, 27). These were cut with safety-razor blades into small pieces, from which samples were taken and analyzed.

From the skinned carcasses the heads, tails, lower leg joints, and abdominal and thoracic organs were removed. The remainder was sawed very carefully into two equal halves down the middle of the back bone. Each of these halves was weighed and placed in the cooler.

The intestinal tract was tied off at each end before it was removed from the body. After it was removed, it was weighed together with its contents. The contents were then washed out, the empty tract weighed, and the weight of the contents obtained by the difference.

Of the organs, the liver, kidneys, and intestinal tract were weighed and analyzed separately. All the other organs and the nervous system were weighed, ground up, and analyzed as one sample, called "miscellaneous organs" in the tables.

Three samples of meat were weighed and analyzed separately; namely, the ninth, tenth, and eleventh rib cut, minus the so-called "eye" from this cut, the "eye", and the round steak from the hind quarter. The greater part of the rest of the meat and fat from one side of the carcass was weighed and analyzed as one sample. Before the carcass was cut up, however, the kidney and bed fat were removed and weighed as a separate sample.

The meat was roughly separated from the bones of the head, tail, and lower leg joints and put into the sample called "miscellaneous meat and fat." The same procedure was followed with the bones from the carcass. The radius and ulna, tibia, metacarpus, metatarsus, and the tenth, eleventh, twelfth, and thirteenth ribs of the carcass bones were carefully cleaned and weighed in the green state. Their weights were compared with the body weights; the results of the com-

parison are given in table 6. The other bones of the carcass were only roughly cleaned with a knife. All the roughly cleaned bones were boiled for some time, after which most of the adhering meat, fat, tendon, etc., was easily removed. This material was added to the "miscellaneous meat and fat", while the bones thus cleaned were united with the carefully cleaned bones, and the whole sample was ground and analyzed. This is the portion called "bones" in table 11.

The hoofs were removed from the lower leg by heating in boiling water, and separately analyzed.

The percentage composition of the parts into which the cows were divided and the percentage composition of the live empty weight and of the fat-free empty weight are given in table 11. The following description will show how these figures were obtained and how they are to be interpreted.

The cows were given no food for 24 hours before slaughter. Each was weighed just before slaughter (p. 10). The live empty weight is obtained by subtracting the weight of the intestinal contents from the live weight just before slaughter. The fat-free empty weight is obtained by subtracting the weight of the total fat contained in the body from the live empty weight.

The figures for the weights of the various parts of the body—bones, hide, and hair, etc.—give the weights of the parts as obtained just before samples were taken out for analysis, except that, in the case of parts taken from one side of the body only, as kidney, carcass meat, etc., these weights are multiplied by two. The figures for the percentage composition of the parts give the composition of the parts as analyzed. The true weight of each constituent—calcium, phosphorus, etc.—of each part is, therefore, obtained by multiplying the weight of the part by the percentage figure and dividing by 100. The percentage of each constituent in the live empty weight and in the fat-free empty weight is obtained by dividing the sum of the weights of that constituent in all the parts by the live empty weight, etc., and multiplying by 100. The figures for the composition of the live empty weight and of the fat-free empty weight, therefore, give the true percentage composition of these parts as constituents of the animal just before slaughter.

The figures for the composition of all of the other parts, except the miscellaneous meat and fat and possibly the bones, are slightly higher than they would be in the live animal, because these parts lost some water in the processes of cooling and mincing or grinding for analysis. It was determined that the carcass lost about 3 percent of its weight during cooling. The situation is altered in the case of the miscellaneous meat and fat and possibly the bones by the fact that the roughly cleaned bones were boiled and allowed to cool under water, and that the soft tissues thus removed from the bones were placed in the portion called miscellaneous meat and fat. The soft tissues adhering to the bones probably took up water during this process, and the figures for the composition of the miscellaneous meat and fat are, therefore, probably lower than they would be in the live animal.

The sum of the weights of all the parts of cow 84 is about 14 kg lower than the live weight just before slaughter, while of the other two cows the live weights are very nearly equal to the sums of the weights of the parts. It seems probable that this is due to the fact that more care was taken to remove the soft tissues from the bones

of cow 84 by means of the knife than in the other two cases. In her case, therefore, the sample called miscellaneous meat and fat is much smaller than in the other two cases, and the taking up of water by the portion of this sample that came from the roughly cleaned bones does not come so near to compensating for the loss of water by the other tissues during cooling.

TABLE 11.—*Weight and percentage composition of body parts into which cows 84, 458, and 499 were divided for study*

COW 84							
Body or body part considered	Weight	Calcium	Phos- phorus	Ash	Nitrogen	Water	Fat
	Kilograms	Percent	Percent	Percent	Percent	Percent	Percent
Live empty weight.....	337.3	1.76	0.93	5.24	3.26	62.91	7.08
Fat-free empty weight.....	313.4	1.90	1.00	5.64	3.51	67.71	-----
Intestinal contents.....	62.3	-----	-----	-----	-----	-----	-----
Bones.....	36.2	16.21	7.47	41.71	3.28	27.05	9.48
Hoofs.....	.7	.083	.055	3.98	10.42	34.04	.21
Hide and hair.....	27.2	.014	.037	.55	5.72	65.75	1.02
Kidney and bed fat.....	2.6	.078	.174	.73	1.86	37.38	50.25
Liver.....	7.5	.008	.311	.90	3.32	74.08	2.95
Kidneys.....	1.4	.013	.214	.90	2.53	79.12	2.90
Intestinal tract.....	26.3	.022	.134	.70	2.31	79.72	5.02
Blood.....	24.5	.0067	.0195	.92	2.61	82.36	.00
Miscellaneous organs.....	38.3	.060	.166	.60	2.26	70.92	14.00
Carass meat.....	133.9	.013	.182	.95	3.51	70.98	5.60
Rib cut minus eye.....	3.2	.012	.174	.70	3.39	68.40	8.74
Eye from rib cut.....	1.5	.0055	.205	1.00	3.60	76.32	1.02
Round steak.....	6.3	.0044	.215	-----	3.61	73.90	2.61
Miscellaneous meat and fat.....	13.6	.144	.234	2.12	4.77	39.96	29.15
COW 458							
Live empty weight.....	343.4	1.62	0.85	4.79	2.85	57.07	20.27
Fat-free empty weight.....	273.8	2.04	1.07	6.01	3.57	71.58	-----
Intestinal contents.....	50.7	-----	-----	-----	-----	-----	-----
Bones.....	33.9	16.22	7.59	41.56	3.15	24.80	10.83
Hoofs.....	.9	.073	.043	.64	11.20	34.66	1.087
Hide and hair.....	26.5	.022	.052	.83	6.19	62.98	1.04
Kidney and bed fat.....	10.6	.0018	.0076	.13	.39	9.60	87.12
Liver.....	6.2	.0090	.359	.37	3.49	71.25	4.46
Kidneys.....	1.3	.0074	.220	1.09	2.38	79.89	2.86
Intestinal tract.....	29.1	.031	.118	.66	1.76	77.05	10.08
Blood.....	21.3	.0067	.0175	.93	2.96	79.78	.00
Miscellaneous organs.....	44.2	.024	.060	.65	1.50	47.48	43.14
Carass meat.....	121.4	.0086	.155	.74	2.92	61.50	19.11
Rib cut minus eye.....	3.4	.016	.097	.65	2.32	47.62	30.62
Eye from rib cut.....	1.1	.0052	.220	1.13	3.60	71.06	4.82
Round steak.....	3.0	.0048	.219	1.05	3.48	72.70	2.90
Miscellaneous meat and fat.....	41.3	.102	.132	.92	2.70	59.15	23.58
COW 499							
Live empty weight.....	389.2	1.25	0.70	3.77	2.50	52.36	29.29
Fat-free empty weight.....	275.2	1.74	.97	5.26	3.49	73.03	-----
Intestinal contents.....	43.4	-----	-----	-----	-----	-----	-----
Bones.....	33.4	14.12	6.89	36.69	2.90	27.10	14.90
Hoofs.....	.9	.063	.036	.52	10.64	34.20	.09
Hide and hair.....	29.0	.016	.036	.81	5.55	67.14	1.24
Kidney and bed fat.....	9.1	.0077	.020	.14	.37	5.67	92.46
Liver.....	5.4	.0045	.372	1.34	3.49	66.96	8.71
Kidneys.....	1.1	.0098	.194	1.12	2.35	78.00	4.08
Intestinal tract.....	26.0	.013	.096	.59	1.94	77.82	11.62
Blood.....	21.1	.0052	.0202	.95	2.98	79.70	.00
Miscellaneous organs.....	52.4	.020	.083	.44	1.10	34.92	55.14
Carass meat.....	167.5	.0068	.118	.61	2.37	48.20	36.94
Rib cut minus eye.....	4.8	.0068	.078	.38	1.70	32.56	56.60
Eye from rib cut.....	1.5	.0052	.205	1.00	3.52	68.60	8.25
Round steak.....	4.6	.0044	.208	1.03	3.61	72.48	3.46
Miscellaneous meat and fat.....	32.4	.094	.115	.62	2.22	77.82	8.95

The results of the analyses in table 11 will be discussed further in connection with the Missouri results, in the general discussion.

EXPERIMENTS IN GROUP 3

BALANCE EXPERIMENTS WITH COWS THAT HAD BEEN FED TIMOTHY RATIONS FOR LONG PERIODS

In work carried out at the Wisconsin and Beltsville Experiment Stations, and reviewed a few years ago (15), the calcium assimilation of cows fed alfalfa and timothy hay ranged from 5 to 28 percent of the intake. The percentage assimilation when fed timothy hay showed no tendency to be higher than when fed alfalfa hay. In the work of Huffman (12) and Ellenberger (4), on the other hand, the calcium assimilation of cows fed timothy hay was often about 50 percent of the intake.

In the Beltsville work, and probably also in the Wisconsin work, the cows had been fed rations containing alfalfa hay and liberal quantities of calcium for long periods before the calcium balances were determined. In Huffman's experiments, on the other hand, and probably also in Ellenberger's, the cows had been fed low-calcium rations for long periods.

In view of the work described in the foregoing part of this paper, it seems probable that these different tendencies to assimilate calcium were due to the fact that the cows which had been fed alfalfa still had a considerable surplus stock of calcium in their bones when the balances were determined, while those which had been fed timothy had reached the bottom of their reserves, or nearly so. But, for a number of reasons, it has seemed worth while to carry out some further experiments on this subject. These reasons may be stated as follows: Work at the Wisconsin (6) and Beltsville (15) Stations indicates that cows which have previously been fed rations high in calcium may continue for considerable periods to lose calcium from their bodies when transferred to rations low in calcium. The cow used in the Wisconsin work apparently lost calcium from her body continuously during a period of 16 weeks in which the balance was determined at intervals. Two cows used in the Beltsville work lost calcium continuously for a period of 19 weeks.

This long-lasting influence of previous treatment may explain many of the apparently discordant results of balance experiments, in most of which little is said about the previous treatment of the animals. It seems justifiable, therefore, to put on record the results of a balance experiment at Beltsville in which this point is taken into account. Three cows were used in the experiment—N105, N216, and N406. Data regarding their history previous to the beginning of the balance experiment are given in table 12. As may be seen from the table, all the cows had been fed low-calcium rations for more than 8 months before the balance experiment started.

TABLE 12.—*Historical record of the three cows used in the balance experiments at Beltsville, Md.*

Cow no.	Date of birth	Put on low-calcium ration	Constituents of ration	Last calving before balance experiment	Balance experiment started
N105.....	Aug. 5, 1927	July 1, 1930	Grain 65, no. 1 timothy hay, corn silage.	May 23, 1931	Mar. 2, 1932
N216.....	Jan. 26, 1929	Apr. 1, 1931	Grain 65, no. 1 timothy hay..	June 21, 1931	Do.
N406.....	Oct. 1, 1928	June 4, 1931do.....	Oct. 18, 1931	Do.

The data pertaining to the balance experiment are given in tables 13 to 16. The balance experiment proper began March 2, 1932. From this time on the rations for all these cows consisted of grain 65 and no. 1 timothy hay, coarsely chopped. As in previous experiments at this station, the output of calcium and phosphorus in the feces and urine of each weekly period is balanced against the intake in the food for a weekly period beginning 2 days earlier, in order to allow for the intestinal lag.

TABLE 13.—*Water, calcium, and phosphorus content of feeds used at different times in the balance experiments*

Feed	Period of feeding	Water	Calcium	Phosphorus
		<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Timothy hay (T 35).....	Mar. 2 to 29.....	10.50	0.280	0.171
Grain 65 (batch 1).....	Mar. 2 to 12.....	11.92	.165	.684
Grain 65 (batch 2).....	Mar. 13 to 22.....	12.16	.166	.663
Grain 65 (batch 3).....	Mar. 23 to 29.....	11.56	.179	.644

TABLE 14.—*Calcium and phosphorus content of the milk from 3 cows in the balance experiments*

Experimental period	Cow N105		Cow N216		Cow N406	
	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Mar. 4 to 10.....	0.092	0.097	0.119	0.098	0.148	0.136
Mar. 11 to 17.....	.091	.096	.122	.101	.149	1.36
Mar. 18 to 24.....	.093	.096	.135	.098	.154	.134
Mar. 25 to 31.....	.094	.095	.120	.106	.150	.132

TABLE 15.—*Average daily feed consumption by 3 cows in the balance experiments*

Experimental period	Cow N105		Cow N216		Cow N406	
	Grain	Hay	Grain	Hay	Grain	Hay
	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>	<i>Kilograms</i>
Mar. 2 to 8.....	6.60	7.43	6.58	5.11	16.60	15.50
Mar. 9 to 15.....	6.60	8.00	7.00	5.50	6.60	6.00
Mar. 16 to 22.....	6.60	8.00	6.50	3.93	6.60	6.00
Mar. 23 to 29.....	6.60	8.00	4.00	3.93	6.60	6.00

¹ Mar. 3 to 8; see text.

One batch of hay (T35) was sufficient for the entire experiment. Three batches of grain were used.

The cows were fed hay and grain twice a day, and were milked three times a day. They had water at will. They were exercised 10 to 15 minutes daily except Sunday.

A mistake was made in taking the aliquot of the first day's feces of cow N406, so the first period for this cow consists of only 6 days. Cow N216 was off feed and showed a reduced milk yield in the third and fourth periods. The 4.81 kg of refused feed taken from her manger during these periods contained 0.393 percent of calcium and 0.597 percent of phosphorus. Her appetite improved and her milk yield increased toward the end of the fourth period. Except for these incidents, the experiment ran smoothly.

TABLE 16.—Average daily milk yield, and calcium and phosphorus intake, output, and balance of the 3 cows in the balance experiment

COW N105

Experimental period	Milk yield	Calcium					Phosphorus					Ca/P ratio in feed			
		Output			Total intake	Balance	Assimilation	Output		Total intake	Balance		Assimilation		
		In urine and feces	In milk	Total				In urine and feces	In milk					Total	
Kg.	Grams	Grams	Grams	Grams	Grams	Percent	Grams	Grams	Grams	Grams	Grams	Percent	Grams	Percent	
First week	11.8	23.8	10.8	34.6	31.7	-2.9	7.9	25.0	48.8	11.4	60.2	57.8	-2.4	9.1	15.7
Second week	11.9	24.3	10.8	35.1	33.3	-1.8	9.0	27.0	48.7	11.4	60.1	58.1	-2.0	9.5	16.3
Third week	11.7	25.7	10.9	36.6	33.4	-3.2	7.7	23.1	47.4	11.3	58.7	57.4	-1.3	9.9	17.3
Fourth week	11.4	28.9	10.7	39.6	34.2	-5.4	5.3	15.5	47.3	10.8	58.1	56.2	-1.9	8.9	15.8
Average	11.7	25.7	10.8	36.5	33.3	-3.2	7.5	122.5	48.1	11.2	59.3	57.4	-1.9	9.3	16.3

COW N216

First week	11.7	14.3	28.2	25.0	-3.2	10.7	42.8	11.5	60.0	53.2	4.6	8.7
Second week	12.3	14.7	29.7	27.0	-2.7	12.3	45.5	12.4	58.5	56.5	10.4	18.4
Third week	7.7	9.0	19.5	17.4	-2.1	8.4	31.0	7.6	38.6	39.1	8.1	20.8
Fourth week	8.5	11.7	21.9	18.2	-3.7	6.5	25.7	9.0	34.7	32.5	6.7	20.7
Average	10.1	12.4	24.8	21.9	-2.9	9.5	37.8	10.1	47.9	45.3	7.5	16.4

COW N406

First week	9.6	15.3	29.5	26.3	-3.2	11.0	41.9	13.0	56.6	54.5	10.9	20.0
Second week	9.5	15.0	29.1	27.7	-1.4	12.6	45.5	12.9	55.6	54.7	12.0	21.9
Third week	9.6	16.7	31.6	27.8	-3.7	11.1	39.9	12.9	55.1	53.9	11.7	21.7
Fourth week	9.6	17.2	31.6	28.6	-3.0	11.4	41.2	12.6	53.9	52.8	11.5	21.8
Average	9.6	16.0	30.4	27.6	-2.8	11.6	42.5	12.9	55.4	54.0	11.5	21.3

1 Based on totals.

As may be seen from table 16 the average calcium assimilation of cows N216 and N406 was between 40 and 50 percent of the intake. There seems every reason to believe that cows may readily assimilate from 40 to 60 percent of the calcium intake when they are kept on low-calcium rations for long periods. The calcium balances were negative throughout, though not very markedly so.

GENERAL DISCUSSION

COMPARISON OF WORK AT BELTSVILLE WITH THAT IN MISSOURI

The figures in table 11 for the composition of the whole bodies of the cows and of the various portions into which their bodies were divided may be compared with those given in Missouri Research Bulletin 55 (18, *tables 1 to 30*). The two sets of figures agree in many respects, and further study shows that much of the apparent disagreement is due to the varying fat content in the whole bodies and portions of the different animals used. It is easy, of course, to calculate the percentages of calcium, phosphorus, ash, etc., in the fat-free bodies and portions of the animals used; and this has been done to some extent in order to obtain the figures shown in tables 17 and 18.

TABLE 17.—*Calcium and phosphorus in the fat-free bodies of cattle and in various fat-free portions of their bodies, as calculated from analyses made at the Beltsville and Missouri Stations*

BELTSVILLE RESULTS

Kind of animal and no.	Whole body		Total soft tissues		Carcass meat		Bones		Blood		Hide and hair	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Cow 84.....	1.90	1.00	0.031	0.153	0.014	0.193	17.91	8.25	0.0067	0.0195	0.014	0.037
Cow 458.....	2.04	1.07	.038	.147	.011	.192	18.19	8.51	.0067	.0175	.022	.053
Cow 499.....	1.74	.97	.029	.119	.011	.187	16.59	8.10	.0052	.0202	.016	.036
Average.....	1.89	1.01	.033	.150	.012	.191	17.56	8.29	.0062	.0191	.017	.042

MISSOURI RESULTS¹

Calves.....	1.45	0.94	0.022	0.186	0.013	0.207	6.61	3.23	0.0070	0.0214	0.054	0.099
Oxen.....	1.84	.98	.017	.143	.014	.169	11.81	5.55	.0065	.0160	.033	.047
Cows.....		1.04		.197		.212		6.86		.0273		.064

¹ The Missouri figures for calves and oxen are calculated from the figures given in Research Bulletin 107 (11, *tables 6 to 23*). Those for calves are averages for the animals 8 months old and younger; those for oxen, averages for the animals 40 months old and older. The Missouri figures for cows are calculated from those given in Research Bulletin 61 (19) and are averages for the 3 cows used.

In table 17 the Beltsville figures for the calcium and phosphorus content of the fat-free bodies and of various portions of the body are compared with corresponding figures calculated from the Missouri data. The figures for the whole body and for the carcass meat⁴ are in close agreement with the corresponding Missouri figures for mature oxen and cows. It has already been shown that the Missouri figures agree with the figures of Lawes and Gilbert (14) for the calcium and phosphorus content of the fat-free bodies of mature cattle, and it may therefore be assumed that these quantities are nearly constant. As the Beltsville figures agree with those established by the two other investigations, it may be supposed that the analyses are satisfactorily accurate.

TABLE 18.—*Calcium and phosphorus content of live empty weights of cows and of all portions of their bodies calculated on the fat-free basis*

Body part	Calcium			Phosphorus		
	Cow 84	Cow 458	Cow 499	Cow 84	Cow 458	Cow 499
	Percent	Percent	Percent	Percent	Percent	Percent
Live empty weight.....	1.90	2.04	1.74	1.00	1.07	0.97
Total soft tissues.....	.031	.038	.029	.153	.147	.149
Bones.....	17.91	18.19	16.59	8.25	8.51	8.10
Horns.....	.083	.074	.064	.055	.043	.036
Hide and hair.....	.014	.022	.016	.037	.053	.036
Kidney and bed fat.....	.157	.014	.102	.350	.059	.265
Liver.....	.008	.006	.005	.324	.376	.407
Kidney.....	.013	.008	.010	.220	.226	.202
Intestinal tract.....	.023	.034	.015	.141	.131	.109
Blood.....	.0067	.0067	.0052	.019	.017	.020
Miscellaneous organs.....	.070	.042	.045	.193	.106	.185
Carcass meat.....	.014	.011	.011	.193	.192	.187
Rib cut minus eye.....	.013	.023	.016	.191	.140	.175
Eye from rib cut.....	.0050	.0055	.0057	.207	.231	.223
Round steak.....	.0045	.0049	.0046	.221	.226	.215
Miscellaneous meat and fat.....	.203	.133	.103	.330	.173	.126

There is some disagreement between the Beltsville figures and the Missouri figures for other portions of the body, particularly the bones. The Beltsville figures would seem to indicate that bones have percentages of calcium and phosphorus about 50 percent higher than those shown by the Missouri figures. This discrepancy is caused by the different methods by which the portion called skeleton or bones was prepared in the two investigations. In the Beltsville work some of the long bones and ribs were very carefully cleaned before being ground for analysis, and the rest of the skeleton was boiled and then stripped of all adhering soft tissue. In the Missouri work, on the other hand, the skeleton was only roughly cleaned by means of the knife, and the sample so prepared, consisting of bone with a good deal of adhering tendon and other soft tissue was ground and analyzed. It may be pointed out that in the Missouri work the portion called skeleton or bones comprised about 50 percent more of the weight of the total fat-free body than in the Beltsville work, and also that the Beltsville figures for the calcium and phosphorus content of the total bones of cows 84, 458, and 499 agree fairly closely with corresponding figures for the carefully cleaned bones of a number of cows given in table 9. Furthermore, the Beltsville figures agree with those given for clean bones by Henderson and Weakley (9).

The difference in procedure in the Beltsville and Missouri investigations, which has just been considered, explains some of the other discrepancies which appear in table 17. In the Beltsville investigation the scrapings from the carefully cleaned bones and the material removed by boiling from the rest of the skeleton were included in the portion called miscellaneous meat and fat. This portion has, therefore, a considerably higher calcium content than any of the other soft tissues. As the miscellaneous meat and fat is included in calculating the composition of the total soft tissues this latter portion has also a rather high calcium content.

⁴The portion described as "carcass meat" in the Beltsville tabulations corresponds to that called "lean and fat" in the Missouri bulletins.

The Beltsville figures for the calcium content of blood are in fairly close agreement with the Missouri figures. For the phosphorus content of the blood, the Beltsville figure lies between the Missouri figure given for oxen (11) and that given for cows (19). This latter figure is certainly unusually high, but it is well known that the phosphorus content of blood is quite variable. The Beltsville figure for the calcium content of hide and hair is decidedly lower than the Missouri figure, but it seems not unlikely that the small calcium content of this tissue may be subject to variations of this order.

TABLE 19.—*Calcium and phosphorus content and the Ca/P ratio for the live-empty weight of individual mature steers and cows calculated on a fat-free basis*

STEERS ¹

Manner of feeding and animal no.	Age at slaughter	Fat-free bodies		
		Calcium	Phosphorus	Ca/P ratio
	Months	Percent	Percent	
High fed				
527.....	40	1.72	0.93	1.85
513.....	44	1.75	.94	1.86
501.....	48	1.78	.95	1.87
Medium fed				
528.....	40	1.84	.99	1.86
502.....	44	1.79	.95	1.88
512.....	48	2.07	1.08	1.91
Low fed				
524.....	40	1.96	1.04	1.88
509.....	44	1.73	.93	1.89
500.....	48	1.87	.99	1.89
Average.....		1.84	.98	1.88

COWS ²

Fed for maintenance: 63.....	71	1.13	
Fattened			
4.....	112	1.03	
43.....	85	1.06	
Average.....		1.04	

¹ The figures for steers are calculated from those given in Bulletin 55 (18, tables 69 to 71) and in Bulletin 107 (11, table 23).

² The figures for cows are taken from Bulletin 61 (19, table 2.)

In table 19 are given the Missouri figures for the calcium and phosphorus content of the fat-free live empty weights of individual mature oxen and cows, which show the ranges of variation and the averages for these quantities under feeding conditions where all the animals had fairly liberal amounts of calcium in their rations. These figures are to be compared with those for the calcium and phosphorus content of the fat-free bodies of the Beltsville cows given in table 18. It will be seen at once that the differences between the calcium and phosphorus content of the Beltsville cows, 458 and 499, lie within the range of variation for the Missouri animals. If there were no other evidence on the subject than the figures given in these two tables, the results might be taken to show that the quantity of calcium contained in the food of a cow over a long period has little or no influence on the calcium and phosphorus content of her body.

It does not seem, however, that this is the most probable interpretation of the results. Cows 458 and 499 were of similar breed and type and of about the same age, and the weights of their fat-free bodies

and of their bones at slaughter were very nearly equal. Further, the results of balance experiments have repeatedly shown that cows may lose considerable amounts of calcium and phosphorus from their bodies and that these losses are likely to be larger when the calcium content of the ration is low.

Finally, the calcium and phosphorus content of cow 458 is near the upper range of the variation in the Missouri work, while that of cow 499 is near the lower range. The more probable interpretation of the results would seem to be that the two cows had about the same calcium and phosphorus content at the beginning of the experimental feeding periods, and that cow 499 lost about 15 percent of her calcium and about 10 percent of her phosphorus in the period during which she was fed timothy hay.

If this interpretation may be accepted as correct, the results suggest that it may be somewhat disadvantageous to feed cows as little calcium as was contained in the ration of cow 499. The fact, that in the same part of the lactation cycle she had 15 percent less calcium in her body than cow 458, might be taken to indicate that her ability to assimilate calcium from her food was put under a slight strain. In the work of Reed and Huffman (22), already referred to, the cows that got bone meal in addition to a basal ration of grain, timothy hay, and corn silage, seemed to do a little better than those that got the basal ration alone, and this gives some further basis for the views outlined above.

In regard to the question of the percentage of calcium assimilated, it makes little difference whether or not it be supposed that cow 499 lost 15 percent of the calcium content of her body in the 5-year experimental period preceding her slaughter. As shown in table 8, her calcium assimilation in this period, on the assumption that she lost no calcium from her body, was 47.6 percent of the intake. If it is assumed that 15 percent of her body calcium must be subtracted from that part of the output in her milk and calves which she was obliged to supply from her food, her calcium assimilation would be 45.8 percent. This result strongly confirms the conclusion to be drawn from the rather incomplete experiments described in the early part of this paper and from the balance experiments of Huffman (12) and Ellenberger (4), namely, that cows which are kept for long periods on rations low in calcium may readily assimilate from 40 to 50 percent of the calcium intake.

Table 18 gives the calcium and phosphorus content of the various portions of the three Beltsville cows calculated on the fat-free basis. Most of the individual tissues of cow 499 show a somewhat lower calcium content than those of cows 84 and 458, which adds strength to the supposition stated above that her low-calcium ration caused some loss of calcium from her body. In most cases, however, the differences are small, and the constancy of the calcium content of some of the portions, namely, the kidneys and muscles, is quite remarkable. The phosphorus content also of most of the soft tissues is remarkably constant.

Table 18 gives an idea of how calcium and phosphorus are lost from the body of a cow on a low-calcium ration. Much the greater portion of the loss comes from the bones, and the low-calcium ration causes so much loss of phosphorus from the bones that the Ca/P ratio is only slightly lower after the losses have occurred than before.

There is no evidence of any general tendency for phosphorus to be stored in the soft tissues, and both the calcium and phosphorus contents of the soft tissues is so small and so constant that the change in the Ca/P ratio in the whole body is almost entirely controlled by that which occurs in the bones. The Ca/P ratios in the bones of cows 84, 458, and 499 were 2.17, 2.14, and 2.05, respectively; those in their fat-free empty weights, 1.90, 1.91, and 1.79. These figures are to be compared with the extremely constant Ca/P ratios given in table 19 for the oxen analyzed at the Missouri Station. They constitute additional evidence for the view that the low-calcium ration of cow 499 had an effect on the calcium and phosphorus content of her body.

The results which have just been discussed confirm results obtained at the Michigan Station (12) and at the Vermont Station (4) which indicate that cows kept on low-calcium rations for long periods may readily assimilate about 50 percent of the calcium intake. They furnish strong reason for believing, however, that those aspects of the Vermont work which indicate that mature milking cows kept on low-calcium rations for long periods may store large quantities of calcium and phosphorus in proportions very different from those ordinarily found in the body, are due to experimental error. They also give reason to believe that the results of many other balance experiments which suggest that milking cows on high-calcium rations are usually losing calcium from their bodies are due either to experimental error or to some peculiarity in the experimental method which renders the results not representative of the usual course of calcium and phosphorus metabolism in long periods of time. The Beltsville results suggest that even on rations low in calcium it is difficult to reduce the calcium content of a cow's body to less than 85 percent of the normal level. In view of the high degree of constancy of the percentages of calcium and phosphorus in the mature fat-free bovine body, and of a number of difficulties in the technic of balance experiments, the method of slaughter and body analysis seems preferable to the balance method, when calcium and phosphorus metabolism is to be studied over long periods.

PRACTICAL CONSIDERATIONS

The practical object of such work as that just described is, of course, to determine the requirements of dairy cows for various nutritional materials, and the pathological effects which are produced by deficiencies of such materials. In 1924, when the experiments were started, there was much evidence on hand to show that feeding low-calcium roughage, such as wheat straw and timothy hay, to dairy cows was likely to cause premature calving and the throwing of dead calves; and many investigators were inclined to attribute these results to the low-calcium content of the roughage. With the passage of time, however, it has become clear that timothy hay and wheat straw are likely to be deficient not only in calcium, but also in vitamin A; and the relative nutritional qualities of these roughages and of alfalfa hay cannot be intelligently discussed without taking this matter into consideration.

Recent work at the writers' station has thrown some light on the vitamin A content of alfalfa and timothy hay. The results may be outlined as follows: The vitamin A content of hay is extremely

variable. There is no doubt, however, that it tends to be higher in alfalfa than in timothy hay, and higher in hay of good quality than in hay of poor quality. It is still too early to give figures which can be regarded as reliable averages, but the results so far obtained may be tentatively taken to indicate that No. 1 alfalfa hay contains about 40 international units of vitamin A activity per gram; No. 3 alfalfa, about 6 units; No. 1 timothy, about 15 units; and No. 3 timothy, about 3 units.

In still other unpublished experiments at the writers' station, cows have been feed for periods of several years on rations of grain combined with each of the above-mentioned kinds of hay as the sole roughage. Of these cows, only those fed on No. 1 alfalfa have reproduced satisfactorily. Those fed on No. 3 alfalfa and on No. 3 timothy have uniformly calved prematurely and thrown weak or dead calves. Those fed on No. 1 timothy have usually thrown weak or dead calves prematurely, but cow 499 has had four normal calves in a period of 5 years on this ration.

The writers have made numerous determinations of the calcium content of the No. 3 alfalfa hay, and find that it is practically the same as that of the No. 1 alfalfa. The results obtained at this station, therefore furnish clear evidence for the view that cows will calve prematurely and will throw dead or weak calves when their rations are deficient in vitamin A, even though such rations may contain adequate quantities of calcium.

As it is clear that a vitamin A deficiency without any accompanying calcium deficiency may cause abnormal reproduction, it seems reasonable to attribute the abnormal calvings of the cows on no. 1 timothy hay to the low vitamin A content of this ration rather than to its low-calcium content, even though the vitamin A content was somewhat higher than that of the No. 3 alfalfa ration.

In the case of cow 499, either because her vitamin A requirement was lower than that of the other cows, or because the vitamin A content of her ration happened to be a little higher, reproduction remained normal throughout the period of experimental feeding. The results obtained in her case may, therefore, be taken as a contribution to the study of rations which are rather low in calcium, and, nevertheless, not sufficiently lacking in vitamin A to cause abnormal reproduction.

During the 5 years of timothy hay feeding, she gave about 2,800 kg of milk annually on a ration which contained 25.1 g of calcium daily. She probably lost about 15 percent of the calcium contained in her body at the beginning of the experiment. The milk yield was somewhat lower than that which has been obtained from cows of similar type on No. 1 alfalfa hay, and the writers judge that it was reduced about 10 percent by the timothy hay feeding. The calcium content of the ration was undoubtedly much lower than that of the rations fed on the average dairy farm throughout this country, but by no means as low as may sometimes occur under conditions which prevail in certain districts. The Beltsville grain mixture contained a large proportion of oil meal, and therefore a much higher calcium content than would a mixture made up of cereals and wheat bran, and the timothy hay had a decidedly higher calcium content than is given by Henry and Morrison (10) as the average for this roughage. The results definitely suggest that an intake of 25 g of calcium daily is rather low for Jersey cows which are fairly good milkers, and it is not at all

impossible that, if the field is carefully surveyed, as has been done in the study of phosphorus deficiencies (3), regions may be found in which the rations contain less calcium than this, and in which calcium deficiency is a serious factor in dairy practice.

Some beneficial results have been obtained by adding calcium salts to rations which were low in their natural calcium content (1; 2, 22). But, in view of the fact that timothy hay is low in vitamin A as well as in calcium, while alfalfa contains more liberal quantities of both of these essentials, it seems wise at the present time to include a large proportion of legume hay of good quality in the winter dairy ration, wherever possible, and thus to insure at the same time against calcium and vitamin A deficiency. The question whether it may be economical, under certain circumstances, to feed timothy hay, and to provide additional calcium and vitamin A separately in the form of such materials as bone meal and carrots, is a matter for future investigation.

SUMMARY

The balance experiments carried out in the past have been rather contradictory and confusing in regard to certain features of calcium and phosphorus metabolism. Some results suggest that milking cows are likely to be in negative calcium balance, even on rations high in calcium, and that they generally do not utilize more than 20 to 30 percent of the calcium intake for the production of milk and calves. Other results suggest that they may often be in positive balance, even on rations low in calcium, and that they frequently utilize from 40 to 60 percent of the calcium contained in such rations for the production of milk and calves. Certain recent results reported from the Vermont Agricultural Experiment Station indicate that liberally milking cows may store large amounts of calcium and phosphorus in the course of several years on rations low in calcium, and that the proportions of calcium and phosphorus stored may be quite different from those which are contained in normal bone.

In order to obtain light on all these questions, the calcium and phosphorus metabolism of cattle has been studied by slaughtering them and making analyses of their bodies and bones after they had been fed for long periods rations containing different amounts of calcium. The results obtained in these body analyses are compared with the normal calcium and phosphorus content of the fat-free bodies of cattle as shown by analyses carried out in the past. The results of this part of the work may be stated as follows:

(1) The percentages of calcium and phosphorus contained in the fat-free bodies of mature normal cattle are quite constant, and the Ca/P ratio, both in the whole body and in bone, is highly constant.

(2) Keeping cows on low-calcium rations for long periods alters the above relationships less than has often been thought. The Beltsville results indicate that after periods of several years on rations containing only about 25 g of calcium daily, the calcium content of the fat-free bodies of dairy cows is not likely to be reduced to less than 85 percent of the normal level. The change in the Ca/P ratio for the whole body produced by such treatment is even smaller, namely, from 1.9 to about 1.8.

(3) Cows which have been fed low-calcium rations for 9 months or more readily utilize about 50 percent of the calcium intake for the

production of milk and calves. This conclusion is confirmed by the results of balance experiments reported in this paper, in which the calcium and phosphorus balances of cows were determined after they had been fed low-calcium rations for long periods and were still on such rations.

(4) There is every reason to believe that the results of certain balance experiments which suggest that cows may store calcium and phosphorus in proportions entirely different from those usually found in the body, are based on experimental error. The conclusions drawn from other experiments to the effect that cows fed rations high in calcium are usually losing calcium from their bodies; and from still others, that cows fed rations low in calcium may sometimes be storing large quantities of calcium, are also either based on experimental error or due to the fact that the results are not representative of what occurs in long periods.

The work in which calcium and phosphorus metabolism have been studied by means of balance experiments is considered in relation to that in which the general health and performance of milking cows have been studied while they were kept for long periods on rations containing different quantities of calcium. It appears that, when the calcium content of a ration is reduced by substituting timothy hay or straw for alfalfa, the vitamin A content is also likely to be reduced, and that the failures in reproduction, which have occurred on rations in which the roughage was timothy hay or straw, are to be attributed to a vitamin A deficiency rather than to calcium deficiency. The physiological effects of rations which are deficient in calcium, though adequate in vitamin A, are in need of further investigation. Some of the results of the present study may be taken as the beginning of such an investigation. They suggest that, for Jersey cows which are capable of giving 3,000 kg of milk or more annually, an intake of 25 g of calcium daily is somewhat inadequate.

LITERATURE CITED

- (1) ANDERSON, B. M., McCAMPBELL, C. W., and ALEXANDER, M. A.
1929. CATTLE FEEDING INVESTIGATIONS, 1928-29. Kans. Agr. Expt. Sta. Circ. 152, 13 pp., illus.
- (2) ——— McCAMPBELL, C. W., and MARSTON, H. W.
1928. CATTLE FEEDING INVESTIGATIONS, 1926-27. Kans. Agr. Expt. Sta. Circ. 143, 13 pp., illus.
- (3) ECKLES, C. H., GULLICKSON, T. W., and PALMER, L. S.
1932. PHOSPHORUS DEFICIENCY IN THE RATIONS OF CATTLE. Minn. Agr. Expt. Sta. Tech. Bull. 91, 118 pp., illus.
- (4) ELLENBERGER, H. B., NEWLANDER, J. A., and JONES, C. H.
1931. CALCIUM AND PHOSPHORUS REQUIREMENTS OF DAIRY COWS. I. WEEKLY BALANCES THROUGH LACTATION AND GESTATION PERIODS. Vt. Agr. Expt. Sta. Bull. 331, 27 pp., illus.
- (5) HAIGH, I. D., MOULTON, C. R., and TROWBRIDGE, P. F.
1920. COMPOSITION OF THE BOVINE AT BIRTH. Mo. Agr. Expt. Sta. Research Bull. 38, 47 pp., illus.
- (6) HART, E. B., McCOLLUM, E. V., and HUMPHREY, G. C.
1909. THE ROLE OF THE ASH CONSTITUENTS OF WHEAT BRAN IN THE METABOLISM OF HERBIVORA. Amer. Jour. Physiol. 24: 86-103.
- (7) ——— STEENBOCK, H., KLINE, O. L., and HUMPHREY, G. C.
1930. DIETARY FACTORS INFLUENCING CALCIUM ASSIMILATION. XIII. THE INFLUENCE OF IRRADIATED YEAST ON THE CALCIUM AND PHOSPHORUS METABOLISM OF MILKING COWS. Jour. Biol. Chem. 86: 145-155.

- (8) HARTMAN, A. M., and MEIGS, E. B.
1931. CALCIUM ASSIMILATION AS INDICATED BY BONE ANALYSIS IN LONG-TIME EXPERIMENTS. *Jour. Dairy Sci.* 14: 322-336.
- (9) HENDERSON, H. O., and WEAKLEY, C. E., JR.
1930. THE EFFECT OF FEEDING DIFFERENT AMOUNTS OF CALCIUM AND PHOSPHORUS UPON THE GROWTH AND DEVELOPMENT OF DAIRY ANIMALS. *W. Va. Agr. Expt. Sta. Bull.* 231, 55 pp., illus.
- (10) HENRY, W. A., and MORRISON, F. B.
1928. FEEDS AND FEEDING; A HANDBOOK FOR THE STUDENT AND STOCKMAN. Rewritten by F. B. Morrison. Ed. 19, unabridged, 770 pp., illus. Ithaca, N. Y.
- (11) HOGAN, A. G., and NIERMAN, J. L.
1927. STUDIES IN ANIMAL NUTRITION. VI. THE DISTRIBUTION OF THE MINERAL ELEMENTS IN THE ANIMAL BODY AS INFLUENCED BY AGE AND CONDITION. *Mo. Agr. Expt. Sta. Research Bull.* 107, 45 pp., illus.
- (12) HUFFMAN, C. F., ROBINSON, C. S., and WINTER, O. B.
1930. THE CALCIUM AND PHOSPHORUS METABOLISM OF HEAVILY MILKING COWS. *Jour. Dairy Sci.* 13: 432-448.
- (13) KATZ, J.
1896. DIE MINERALISCHEN BESTANDTHEILE DES MUSKELFLEISCHES. *Pflüger's Arch. Physiol.* 63: 1-85, illus.
- (14) LAWES, J. B., and GILBERT, J. H.
1859-83. EXPERIMENTAL INQUIRY INTO THE COMPOSITION OF SOME OF THE ANIMALS FED AND SLAUGHTERED AS HUMAN FOOD. *Roy. Soc. London, Phil. Trans.* 149: 493-680, 1859; 174: 865-890, 1883 (pp. 865-890 are sup. to pp. 493-680).
- (15) MEIGS, E. B., TURNER, W. A., HARDING, T. S., HARTMAN, A. M., and GRANT, F. M.
1926. CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS. *Jour. Agr. Research* 32: 833-860, illus.
- (16) MOULTON, C. R., TROWBRIDGE, P. F., and HAIGH, L. D.
1921. STUDIES IN ANIMAL NUTRITION. I. CHANGES IN FORM AND WEIGHT ON DIFFERENT PLANES OF NUTRITION. *Mo. Agr. Expt. Sta. Research Bull.* 43, 111 pp., illus.
- (17) ——— TROWBRIDGE, P. F., and HAIGH, L. D.
1922. STUDIES IN ANIMAL NUTRITION. II. CHANGES IN PROPORTIONS OF CARCASS AND OFFAL ON DIFFERENT PLANES OF NUTRITION. *Mo. Agr. Expt. Sta. Research Bull.* 54, 76 pp., illus.
- (18) ——— TROWBRIDGE, P. F., and HAIGH, L. D.
1922. STUDIES IN ANIMAL NUTRITION. III. CHANGES IN CHEMICAL COMPOSITION ON DIFFERENT PLANES OF NUTRITION. *Mo. Agr. Expt. Sta. Research Bull.* 55, 88 pp., illus.
- (19) ——— TROWBRIDGE, P. F., and HAIGH, L. D.
1923. STUDIES IN ANIMAL NUTRITION. V. CHANGES IN THE COMPOSITION OF THE MATURE DAIRY COW WHILE FATTENING. *Mo. Agr. Expt. Sta. Research Bull.* 61, 20 pp.
- (20) NEAL, W. M., PALMER, L. S., ECKLES, C. H., and GULLICKSON, T. W.
1931. EFFECT OF AGE AND NUTRITION ON THE CALCIUM PHOSPHATE/CALCIUM CARBONATE RATION IN THE BONES OF CATTLE. *Jour. Agr. Research* 42: 115-121.
- (21) PARKER, E. C., and SEEDS, K. B.
1925. HANDBOOK OF OFFICIAL HAY STANDARDS. U. S. Dept. Agr., Bur. Agr. Econ. Form H F S-540, 48 pp.
- (22) REED, O. E., and HUFFMAN, C. F.
1930. THE RESULTS OF A FIVE-YEAR MINERAL FEEDING INVESTIGATION WITH DAIRY CATTLE. *Mich. Agr. Expt. Sta. Tech. Bull.* 105, 63 pp., illus.
- (23) RITCHIE, W. S., MOULTON, C. R., TROWBRIDGE, P. F., and HAIGH, L. D.
1923. STUDIES IN ANIMAL NUTRITION. IV. THE NITROGEN, ASH AND PHOSPHORUS DISTRIBUTION IN BEEF FLESH AS AFFECTED BY AGE AND CONDITION. *Mo. Agr. Expt. Sta. Research Bull.* 59, 78 pp.
- (24) SHERMAN, H. C., and MACLEOD, F. L.
1925. THE CALCIUM CONTENT OF THE BODY IN RELATION TO AGE, GROWTH, AND FOOD. *Jour. Biol. Chem.* 64: 429-459, illus.

- (25) SHERMAN, H. C., and QUINN, E. J.
1926. THE PHOSPHORUS CONTENT OF THE BODY IN RELATION TO AGE, GROWTH, AND FOOD. *Jour. Biol. Chem.* 67: 667-677, illus.
- (26) SMALL, F. H.
1921. SAMPLING OF LEATHER AND ITS PREPARATION FOR ANALYSIS—COMMITTEE REPORT, 1921. *Jour. Amer. Leather Chem. Assoc.* 16: 394-430, illus.
- (27) ———
1922. PROPOSED PROVISIONAL METHOD FOR SAMPLING LEATHER. *Jour. Amer. Leather Chem. Assoc.* 17: 150-151, illus.
- (28) TURNER, W. A., and HARTMAN, A. M.
1929. CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS. III. THE ADEQUATE RATION FOR HIGH PRODUCING COWS AND THE EFFECT OF EXERCISE ON CALCIUM, PHOSPHORUS AND NITROGEN BALANCES. *Jour. Nutrition* 1: 445-454, illus.

RESPONSE OF THE WOODY PLANTS HIBISCUS SYRIACUS, MALVAVISCUS CONZATTII, AND BUGINVILLEA GLABRA TO LENGTH OF DAY¹

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INTRODUCTION

Since 1920 an extensive literature has developed, showing the responses of a great variety of plants to length of day. For the most part the small annual or perennial herbaceous plants have been used, and very little work has appeared to illustrate the flowering response of the woody plants except in the case of the poinsettia (*Euphorbia pulcherrima* Willd.). It is a relatively simple matter to handle the smaller herbaceous plants, whose blooming responses are usually shown during the first or second year from seed. The problem becomes much more difficult when the larger trees and shrubs are considered, for the small container and the usual type of convenient small dark chamber suitable for the small herbaceous plants will not serve for the woody plants.

The shrubs chosen for this study were the turkscap hibiscus (*Malvaviscus conzattii* Greenman), the common garden shrub-althea (*Hibiscus syriacus* L.), and bougainvillea (*Buginvillea glabra* Choisy). The shrub-althea has shown itself well adapted to a study of length of day in all respects. It flowers the first or second season from seed, and is readily grown and handled in small containers. The bougainvillea is also highly responsive to length of day, flowering quickly in summertime in response to artificially shortened daylight exposures.

METHODS

It was at first thought that the flowering responses of shrub-althea could be readily determined by the localization method, which required the construction of small ventilated dark cases over branches of flowering individuals long established in the soil out of doors. In 1929 such a case was constructed over a branch at the Arlington Experiment Farm, Rosslyn, Va., and 10 hours of daylight each day were given this portion of the plant before visible budding occurred. The treated portion of the plant, however, flowered at the same time as the portion receiving the full length of day. Since even in the annuals, flowering, when once initiated, often tends to persist for some time after the original stimulus of a suitable length of day has been removed, it was thought best to grow the shrub-althea under the various lengths of day from the seedling stage.

Seed sown in a flat October 15, 1930, remained in a coldframe out of doors throughout the winter. The seed germinated April 20, 1931, and the seedlings were potted to thumb pots May 28 and to large

¹ Received for publication Feb. 28, 1935; issued August 1935.

buckets June 19, 3 to 4 seedlings being placed in each bucket. The tests began June 19, the plants receiving 10, 12, 12.5, 13, 13.5, 14, and 14.5 hours of daylight and full daylight each day. Large ventilated dark houses were used to reduce the daily light-exposure periods to 14.5 hours or less. On March 1, 1932, the plants were separated and were grown singly in buckets to afford more room for each individual. The buckets for the various tests were numbered and the responses of the plants to the lengths of day originally selected were observed for several consecutive years.

The turkscape hibiscus was grown from cuttings made January 7, 1932. These when rooted were transferred to 3-inch pots February 1 and to 4-inch pots March 8. On April 15 they were transferred to buckets, a single plant being grown in each bucket. The tests with the plants receiving 10, 12, 12.5, and 13 hours of daylight daily began May 9, when the plants were 18 inches tall. The tests with 13.5, 14, and 14.5 hours of daylight each day began May 18, when the daylight period from sunrise to sunset had increased sufficiently. This woodyd hibiscus, being a tender plant, was grown in a warm greenhouse averaging 70° to 75° F. until the tests began out of doors.

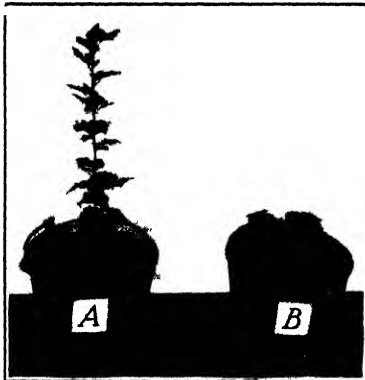


FIGURE 1.—Shrub-althea seedlings 112 days after beginning of tests, which were started May 26, 1930, while plants were in cotyledon stage: A, Control receiving full daylight; B, plants exposed to 10 hours of daylight each day. Photographed September 15, 1930.

another 12 hours of light, and a third was given the full length of day.

EXPERIMENTAL RESULTS

SHRUB-ALTHEA

From the data of table 1 it is obvious that a daily exposure to 10 hours of daylight is too short to allow the shrub-althea to flower, even though the plants have remained for four successive seasons under these conditions. The plants grew very slowly and during the first season remained practically in the rosette condition, forming a very short woody stem only a few inches high upon which thick and abnormally dark green leaves were crowded, as shown in figure 1.

Daily exposure for 12 hours favored greater elongation of the stem, but even this length of day was distinctly unfavorable to flowering. During the seasons of 1931, 1932, 1933, and 1934, only two plants were able to flower, these flowering in 1932, one plant producing a single blossom; the other, three blossoms.

TABLE 1.—Flowering behavior and height of shrub-althea (*Hibiscus syriacus*) in four successive seasons under different daily periods of exposure to light from the seedling stage, the daily light exposure for each plant remaining the same throughout the experiment

Daily light exposure	Plant no	1932					1933					1934				
		Buds ¹	First bloom	Height Inches	Flowers Number	Buds ²	First bloom	Height Inches	Flowers Number	Buds ²	First bloom	Height Inches	Flowers Number	Buds ²	First bloom	Height Inches
10 hours.	1			15				21				23				
	2			12				16.5				20				
	3			15				23				28				
	4			24				14				(¹)				
12 hours.	1			(¹)				39		(¹)		34				
	2	June 22	July 18	25	1			33				34				
	3	June 3	June 24	25	3			32				32				
	4	June 20	July 18	26	5			31				30				
12.5 hours.	1	June 10	July 8	21	10			23				20				
	2	June 10	July 8	21	10			23				20				
	3	June 14	July 11	37	30			45				47				
	4	do	(⁶)	(⁶)	1			40				(¹)				
13 hours.	1	do		40	14			44				48				
	2	do	July 11	31	21			39				41				
	3	June 13	July 8	41	16			45				48				
	4	do	July 8	23	37			33				43				
13.5 hours.	1	July 8	Aug. 6	36	34			46				50				
	2	July 14	July 7	36	34			43				40				
	3	June 1	June 25	45	60			39				51				
	4	June 20	July 7	35	43			44				53				
14 hours.	1	June 9	July 18	30	32			37				49				
	2	June 15	July 5	29	23			40				55				
	3	June 1	July 7	23	6			(¹)				58				
	4	June 21	July 5	48	64			49				51				
14.5 hours.	1	June 5	July 25	43	33			50				56				
	2	June 21	July 25	25	42			(¹)				51				
	3	June 6	June 17	33	10			(¹)				53				
	4	July 21	Aug. 17	44	27			(¹)				47				
Full daylight.	1	June 21	July 22													
	2	June 5	July 25													
	3	June 21	July 25													
	4	June 6	June 17													

¹ The data for 1931 are as follows: Plant 1, under 13 hours of daylight, budded Sept. 5, showed first bloom Oct. 5, was 28 inches in height, and had 1 flower; plants 1 to 4, under 10 hours of daylight, attained a height of 3 to 4 inches, but did not flower and were not measured; none of the other plants flowered and none was measured.

² Leaders indicate that the plants did not flower.

³ Plant died.

⁴ Not measured.

⁵ Many flowers.

⁶ Not recorded.

⁷ Did not flower; plant unhealthy.

Daily exposure for 12.5 hours allowed flowers to develop, but the plants grew relatively slowly and did not attain a floriferous condition until 1933. A rather weak flowering impulse was evident in 1932. Daily periods of exposure of 13, 13.5, 14, and 14.5 hours were quite favorable to growth and flowering, and about as many flowers were produced as appeared under conditions of full daylight. The mean date of appearance of the first flower for the three seasons 1932, 1933, and 1934 was not materially changed under the different periods of exposure favorable to flowering. For the daily 12-hour exposure the mean date of flowering (which occurred only in 1932) was July 6; for the 12.5-hour day, July 21; for the 13-hour day, July 26; for the 13.5-hour day, July 27; for the 14-hour day, July 27; for the 14.5-hour day, August 2; and for full daylight, July 28.

Growth in height showed a consistent increase with increase in the length of the daily period of illumination. The mean height at the end of 1934 for the 10-hour period of exposure was 23.6 inches; for the 12-hour, 34 inches; for the 12.5-hour, 35.5 inches; for the 13-hour,

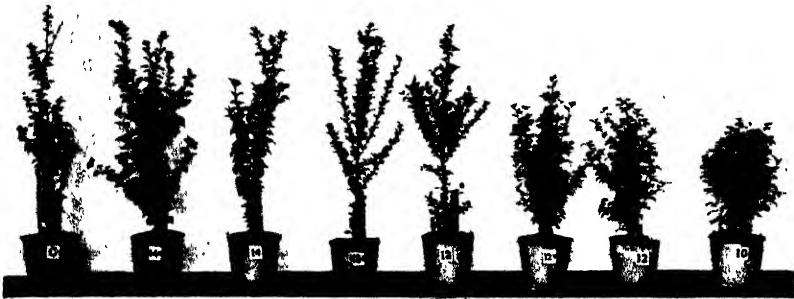


FIGURE 2—Shrub-althea seedlings grown four seasons under the various daily periods of light exposure indicated. Plant designated C received full length of day. Dates of flowering in 1934 under the various periods of exposure: Full day, August 4, 14.5 hours, August 10; 14 hours, August 10, 13.5 hours, August 2; 13 hours, July 26; and 12.5 hours, July 20, 12 and 10 hours, no flowering. Photographed August 7, 1934.

47.5 inches; for the 13.5-hour, 45.5 inches; for the 14-hour, 44.0 inches; for the 14.5-hour, 52.0 inches; and for the conditions of full daylight, 45.0 inches. Figure 2 shows the habit of growth of shrub-althea, under the various daily periods of exposure, at the end of four seasons.

TURKSCAP HIBISCUS

From the data of table 2 it is obvious that the turkscap hibiscus is indeterminate in its responses to length of day, at least for the annual range in day length at Washington, D. C., since it flowered readily under all periods of daylight exposure, from 10 hours to the local full length of day, which on June 21 is about 15 hours from sunrise to sunset. This behavior is illustrated in figure 3. There was some delay in the flowering of some of the plants as the daily periods of exposure to daylight were increased beyond 10 hours. In the initiation of buds the greatest delay occurred under full length of day. Elongation of the stems tended to increase with increase in the length of the daily exposure, the full length of day producing the tallest plants. The plants were more compact and branching in habit of growth under the daily exposure periods of 10, 12, 12.5, and 13 hours. These plants also flowered more freely.



FIGURE 3.—Turkscap hibiscus. A woody subtropical plant flowering freely under all lengths of day, from 10 hours or less to the full length of day, at Washington, D. C. The plants were grown from cuttings made January 7, 1932. Tests for the daily exposure periods of 10, 12, 12.5, and 13 hours began May 9; for periods of 13.5, 14, and 14.5 hours, May 18. The control (C) received the full length of day. The number of days required for budding and first flowering are shown in table 2. Photographed July 18, 1932.

TABLE 2.—Number of days required for production of visible buds and first flowering by turkscap hibiscus (*Malvaviscus conzattii*) and the height attained by the plants when exposed to various day lengths, beginning in May 1932

Daily light exposure	Days required for production of—		Height of plants	Daily light exposure	Days required for production of—		Height of plants
	Buds	First flowers			Buds	First flowers	
	Number	Number			Inches	Number	
10 hours.....	29	54	31	13 5 hours.....	23	54	42
12 hours.....	32	63	37	14 hours.....	28	54	47
12 5 hours.....	32	63	42	14 5 hours.....	28	57	44
13 hours.....	32	63	44	Full day.....	50	63	55

BOUGAINVILLEA

Whereas the shrub-althea behaved as a typical long-day plant, the bougainvillea flowered best in the short daily light periods. Growth of new shoots in the latter began at once in all the tests. The plant receiving 10 hours of daylight each day showed buds June 28, and flowered July 9 at a height of 75 inches. The plant was very floriferous, and quite as gorgeous with brilliant pink flowers as it has been in February when it flowered in response to the natural short days of the winter time. The plant receiving 12 hours of daylight each day budded July 25 and flowered August 4 at a height of 82 inches and was much less floriferous than the plant receiving 10 hours of daylight each day. The controls showed no indications of budding in early October. This behavior of flowering in summertime in response to shortened daily periods of exposure is shown in figure 4. Bougainvillea normally flowers at Washington, D. C., in the greenhouse in December when the length of day from sunrise to sunset is less than 10 hours.

DISCUSSION

Shrub-althea, a woody plant, behaves as a typical long-day plant when grown from the seedling stage and subjected to different daily periods of exposure to daylight in summertime, since flowering is favored by an increasing length of day. A daily exposure of 10

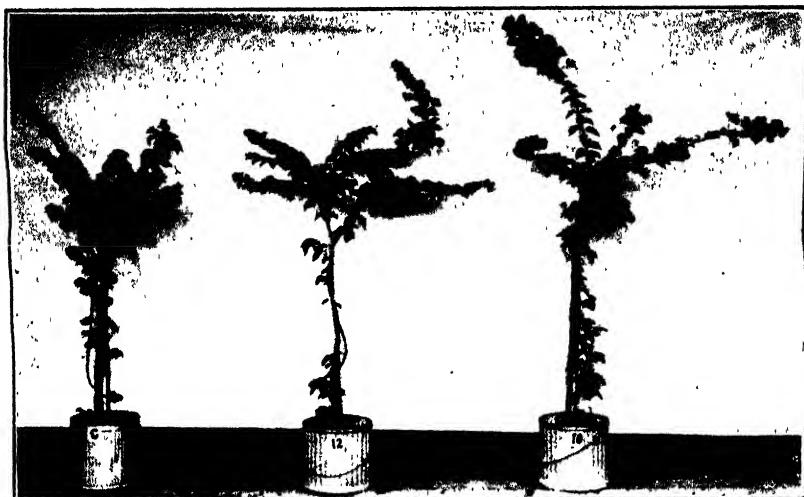


FIGURE 4.—*Bougainvillea glabra* grown with 10 and 12 hours of daylight each day and with full length of day (C). The tests began May 16, 1934, at which time the plants were cut back to 46 inches. The plant receiving 10 hours of daylight each day budded June 28, and flowered July 9 at a height of 75 inches; the plant receiving 12 hours of daylight each day budded July 25, and flowered August 4 at a height of 82 inches; the plants receiving the full daylight had not budded in late October. Flowering normally takes place in December, as shown in figure 5. Photographed August 7, 1934.



FIGURE 5.—*Bougainvillea* flowering in the greenhouse in response to the short days of the wintertime. The first flowers appeared in December 1933 and the plant was in full bloom February 1, 1934. This is one of the plants shown in figure 4 and was induced to flower a second time by reducing the length of day. Photographed February 16, 1934.

hours entirely inhibits flowering and greatly retards growth. The plants grew slowly, being compact and much branched, with a very stocky main stem and short thick branches. A daily exposure of 12 hours is distinctly unfavorable to flowering, but growth is improved.

The turkscap hibiscus is decidedly indeterminate in its flowering response, since it flowers under all lengths of day from 10 hours of daylight to the full length of day at Washington, D. C. For this reason it finds a place as a useful garden ornamental in Florida and other warmer parts of the United States. It flowers readily far northward in summertime, and continues to be floriferous under greenhouse conditions in wintertime. However, being a tender shrub, it is not suitable for outdoor culture where severe winter weather prevails.

Bougainvillea, flowering best on a shortened length of day, behaves as a typical short-day plant. This is indicated by its natural tendency to flower only in midwinter, when the days are shortest, and from the fact that it remains in a flowerless vegetative condition in summertime. In this latitude a day length of only 10 hours is not attained until November 16. The winter flowering behavior is shown in figure 5.

These tests with shrub-althea and the tropical bougainvillea indicate that both woody plants are quite as sensitive to different lengths of day as those annual and perennial herbaceous plants which have been classed as typical long-day or short-day plants.

The poinsettia, also a woody plant, early received attention.² It is obvious that the bougainvillea and the poinsettia have a very similar type of behavior, both flowering when the days are very short. They may be regarded as typical short-day woody plants, since both flower on a shortened length of day. Both are native to the tropical and subtropical regions of the Americas, the bougainvillea being a native of Brazil, the poinsettia of the warmer parts of Mexico and Central America. From the fact that their flowering responses are rigidly dependent upon daily exposures to daylight for 12 hours or less, these plants normally have no horticultural value as flowering plants in northern latitudes except under warm greenhouse conditions in wintertime.

The shrub-althea, a long-day plant, attains flowering on a lengthened day, and because it is floriferous when the summer days are very long becomes useful as a garden ornamental in far-northern latitudes in summertime. A plant which is useful both in the Tropics and under outdoor conditions in high latitudes must be of the indeterminate type with respect to length of day.

SUMMARY

Shrub-althea (*Hibiscus syriacus* L.) behaves in response to length of day as a typical long-day plant, i. e., it flowers in response to a lengthened day. Exposure to 10 hours of daylight each day does not allow flowering. Daily exposure for 12 hours is unfavorable to flowering. Flowering takes place under all lengths of day from 12 or 12.5 hours to and including at least the full length of day of 15 hours in the Washington, D. C., region, but abundant flowering is associated

² GARNER, W. W., and ALLARD, H. A. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871-920, illus. 1923.

with the longer periods of exposure. Growth and elongation are also favored by the longer daily exposure.

The turkscap hibiscus (*Malva viscus konzattii* Greenman) is indifferent or indeterminate in its response to different lengths of day within the range covered by the tests. The plants are more compact in habit of growth and more floriferous under the shorter daily exposure periods, i. e., 10, 12, 12.5, and 13 hours.

Bougainvillea (*Buginvillea glabra* Choisy) behaves as a typical short-day woody plant, flowering on shortened lengths of day. Even in midsummer, when the plants are strictly vegetative, flowering is quickly initiated by daily exposure to 10 hours of daylight. Under the same conditions exposure to 12 hours of daylight each day was distinctly less favorable to flowering.

The day-length requirements of the woody plants shrub-althea, bougainvillea, turkscap hibiscus, and poinsettia are now fairly well understood. The shrub-althea, bougainvillea, and poinsettia are quite as sensitive to the factor of length of day as any of the herbaceous plants. It is evident that the usefulness of all these plants for certain seasons and regions depends as much upon their particular length-of-day requirements as upon any other factor.

PLEOSPORA ROT OF TOMATOES¹

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INTRODUCTION

In the investigation of the various diseases causing losses of fruits and vegetables during transit, storage, and marketing, a new or unusual fungus is sometimes encountered on produce from a more or less localized district. Whether the causal organism is just becoming established in that locality, whether certain new host relationships account for the initiation of the disease, or whether the immediate climatic conditions happened to favor the growth of the organism is often difficult to determine. Pleospora rot of tomato (*Lycopersicon esculentum* Mill.) furnishes an interesting example of a disease induced by an organism known to have been present in the Salinas district of California for at least 11 years without causing serious damage, but later, for reasons not understood, becoming an important factor in the shipping of tomatoes from that district.

In November 1919, a few tomatoes showing an unusual type of decay were obtained from cars of California tomatoes received on the Chicago market. At that time the disease was of little economic importance but was of scientific interest since a *Pleospora* with an associated *Macrosporium* was isolated from the advancing edge of numerous lesions, and a search through the literature failed to reveal any mention of a pleospora rot of tomatoes. Cultures of this fungus have been kept in stock since that time, and each year during November and December other cultures of the organism have been isolated from tomatoes showing similar symptoms on arrival at the Chicago market.

For the past 3 years this disease has been of great economic importance to the California tomato crop grown for marketing during the early winter months. So far as has been observed the disease is not important in those tomato-growing districts of California that ship in other seasons of the year, and it has not been found elsewhere except in Mexican stock shipped during January. Within the past 2 years a few specimens of pleospora rot have been obtained from shipments of Mexican tomatoes arriving on the Chicago and New York markets.

Two hundred and forty-eight isolations of species and strains of *Alternaria* and *Macrosporium* have been made from tomatoes received from practically all of the tomato-producing sections of the country; but, with the exception of the *Macrosporium* having the *Pleospora* stage herein reported, none of them have developed the perfect stage either on the fruit or in pure culture. There are numerous reports of *Alternaria* and *Macrosporium* species associated

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with various types of decay in tomatoes. As indicated by Ramsey and Link (7),² many of these organisms are found following other diseases such as phoma rot, blossom-end rot, and sun scald, while others appear primarily as wound parasites following growth cracks or mechanical injuries, or in the stem scar. These fungi are especially destructive to tomatoes during long transit periods, on track, or in the ripening rooms on the receiving markets.

McWhorter (4) in Virginia and Samuel (8) in South Australia have described a stem-end rot of tomatoes induced by *Macrosporium solani* Ell. and Mart., and Douglas (2) in California reported a new alternaria spot of tomatoes caused by an organism which he believed differed from both *M. solani* and *M. tomato* Cke. The types of lesions described in these reports are similar in some respects to those produced by the *Macrosporium* stage of the *Pleospora* isolated from California tomatoes. However, no perfect stage was observed by the investigators mentioned, and a study of the conidia readily shows the difference between these fungi and the one described in the present paper.

ECONOMIC IMPORTANCE

The best idea as to the seriousness of pleospora rot of tomatoes may be obtained from a study of the inspection reports made by the Food Products Inspection Service of the Bureau of Agricultural Economics, United States Department of Agriculture, on the condition of tomatoes arriving on the larger markets. From these reports it is apparent that the amount of decay induced by *Pleospora* varies greatly from season to season and also with the length of the haul. Decay develops slowly in green tomatoes during the first 3 or 4 days in transit and then makes more and more rapid headway as the fruits begin to ripen. Consequently, the tomatoes arriving on the New York market and other eastern markets often show from 10 to 25 percent more pleospora rot than similar stock received on the Chicago market.

Upon arrival in New York in November 1931, a car of California tomatoes showed 90 percent of the fruit infected at the stem end with *Pleospora*. Another car of tomatoes arrived on this market showing only 2 percent decay, but after the tomatoes had been allowed to ripen in the car on the tracks for almost 2 weeks 50 to 60 percent of them showed serious decay induced by *Pleospora*. A car of California tomatoes that passed shipping-point inspection, containing 85 percent U. S. No. 1 tomatoes with no appreciable decay, was found to have 25 to 80 percent, or an average of 55 percent, pleospora rot when the stock was finally marketed in New York. Numerous cars of California tomatoes shipped during November and December in the last 3 years have shown from 25 to 50 percent loss on account of this disease. The mature, good-quality stock ripens during transit or soon enough after arrival to escape great loss, but the immature or poor-quality stock, which ripens slowly and must often be held for a week or more for ripening, is subject to very heavy decay.

² Reference is made by number (italic) to Literature Cited, p. 42.

THE DISEASE

DEVELOPMENT AND SPREAD

No study has been made of the development and spread of pleospora rot in the field; consequently, no data are at hand concerning its prevalence on growing tomato plants. From studies of the early stages of the disease as found in commercial shipments received on the Chicago market and from correspondence with Federal-State shipping-point inspectors, it is evident that close observation is necessary to detect the presence of early infections about the stem scar of green tomatoes. It appears probable that the fruit pedicels and calyx sometimes harbor the fungus and that under favorable moisture and temperature conditions the organism works its way from this source down into the fruit. In wet weather or during fogs spores lodging under the calyx may also germinate and penetrate the tissues at the edge of the stem scar before the fruit is harvested, or follow immediately in wounds incident to harvesting. There is no evidence that the fungus spreads through the wrappers from one tomato to another during transit.

SYMPTOMS

Small brown V-shaped to oval spots at the edge of the stem scar or in mechanical wounds elsewhere on the fruit are the first visible symptoms of pleospora rot (pl. 1, *A, B*). These lesions appear somewhat similar to those produced by *Phoma*, but as they enlarge the brown color is retained and usually a small amount of gray to grayish-brown mycelium is noticeable over the lesion, whereas in typical *Phoma* lesions of the same size the affected tissues are black and there is no visible surface mold. Spots one-half inch or more in diameter generally show the black pimplelike perithecia forming in the central region. Tomatoes arriving on the market after a 5- to 8-day transit period show lesions varying from one-fourth to three-fourths of an inch in diameter. As the tomatoes ripen the fungus progresses rapidly, and a moderately firm greenish-brown to brown decay may involve one-fourth of a fruit within 10 days after it is shipped (pl. 1, *B, C*). The greatest losses are sustained in shipments of tomatoes that must be held on track or in the humid ripening rooms for several days before they ripen enough to be placed on the market. Under these humid conditions there is usually an abundant development of mycelium which characterizes the *Macrosporium* stage of this fungus (pl. 1, *C, E*). The black perithecia of *Pleospora lycopersici* are prominent in the older lesions (pl. 1, *E*).

Typical *Pleospora* lesions show evidence that the fungus entered through or at the edge of the stem scar. The organism penetrates into the fruit one-half inch or more, causing a moderately firm dark-brown decay such as that shown in plate 1, *D*, and in many instances it discolors the vascular elements underneath the scar and along the side walls of the fruit. When infection takes place through wounds on the sides of the tomato the vascular elements are not discolored.

INOCULATION EXPERIMENTS

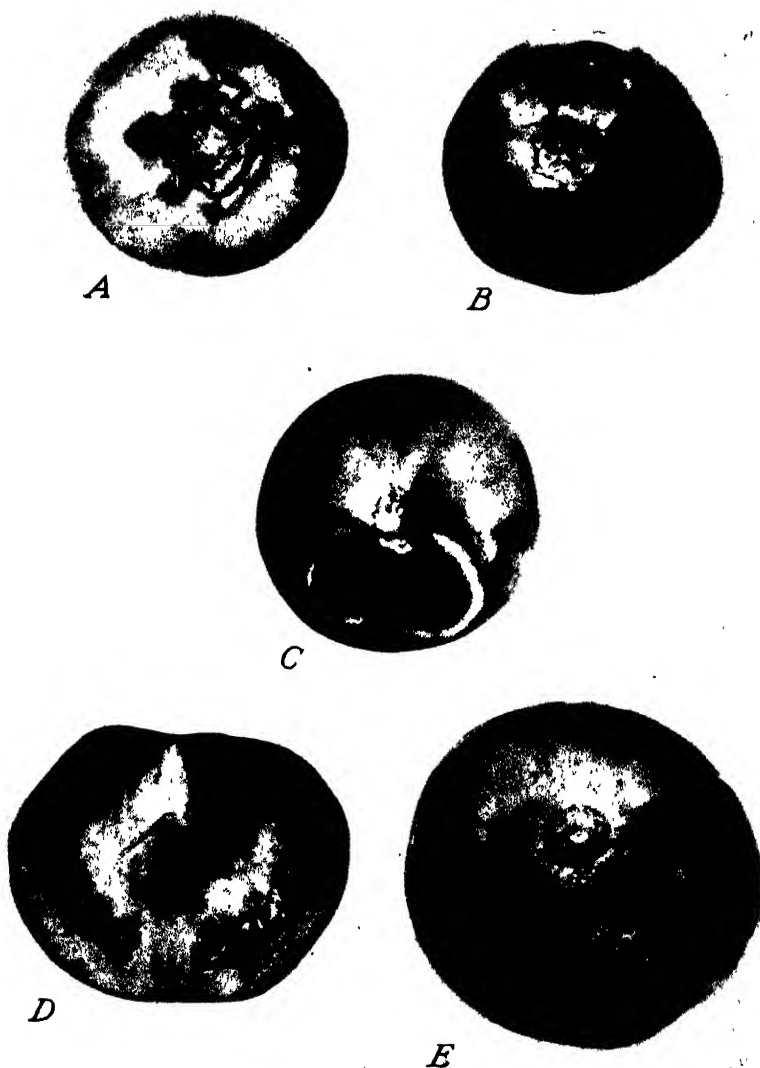
The pathogenicity of both the *Pleospora* and *Macrosporium* stages of this fungus is readily established by inoculation experiments. Mature-green tomatoes, selected for freedom from disease and blem-

ishes, were sterilized in a solution of formaldehyde (1 to 240) for 3 minutes and then rinsed in sterile water before inoculations were made. In each experiment several tomatoes were inoculated by placing mycelium and spores in small punctures through the skin of each fruit. The results obtained from such experiments show that the decay induced progresses slowly in green tomatoes and that as the fruits ripen the development of the lesions becomes more and more rapid. Changes in the chemical composition of the fruit during ripening influence the rate of growth of the fungus. Changes in acidity of the fruit juices probably play an especially important part. Commercially mature-green tomatoes are somewhat acid (pH 4.7), while tomatoes ripe enough for table use are much less acid (pH 6.01).

Mature-green tomatoes inoculated as described above and held at 45° F. develop lesions that can barely be detected within 10 days, and at 55° the lesions reach an average diameter not much greater than one-fourth of an inch in 10 days. The fungus becomes more active at 65°, and lesions one-fourth of an inch in diameter are produced in a week and lesions five-sixteenths of an inch in diameter within 10 days. On cutting the fruit held under these conditions it was found that the average diameter of the lesions within the locules was one-half inch. At 70°, the temperature at which most tomatoes are held for ripening, the average internal diameter of lesions developed in 10 days is about three-eighths of an inch, whereas the external lesions are about one-fourth of an inch. The rate of enlargement of lesions begins to slow down at 75°, few spots reaching a diameter greater than one-fourth of an inch and a depth of five-sixteenths of an inch within 10 days. In mature-green tomatoes held at 85° no development of decay could be detected in 10 days.

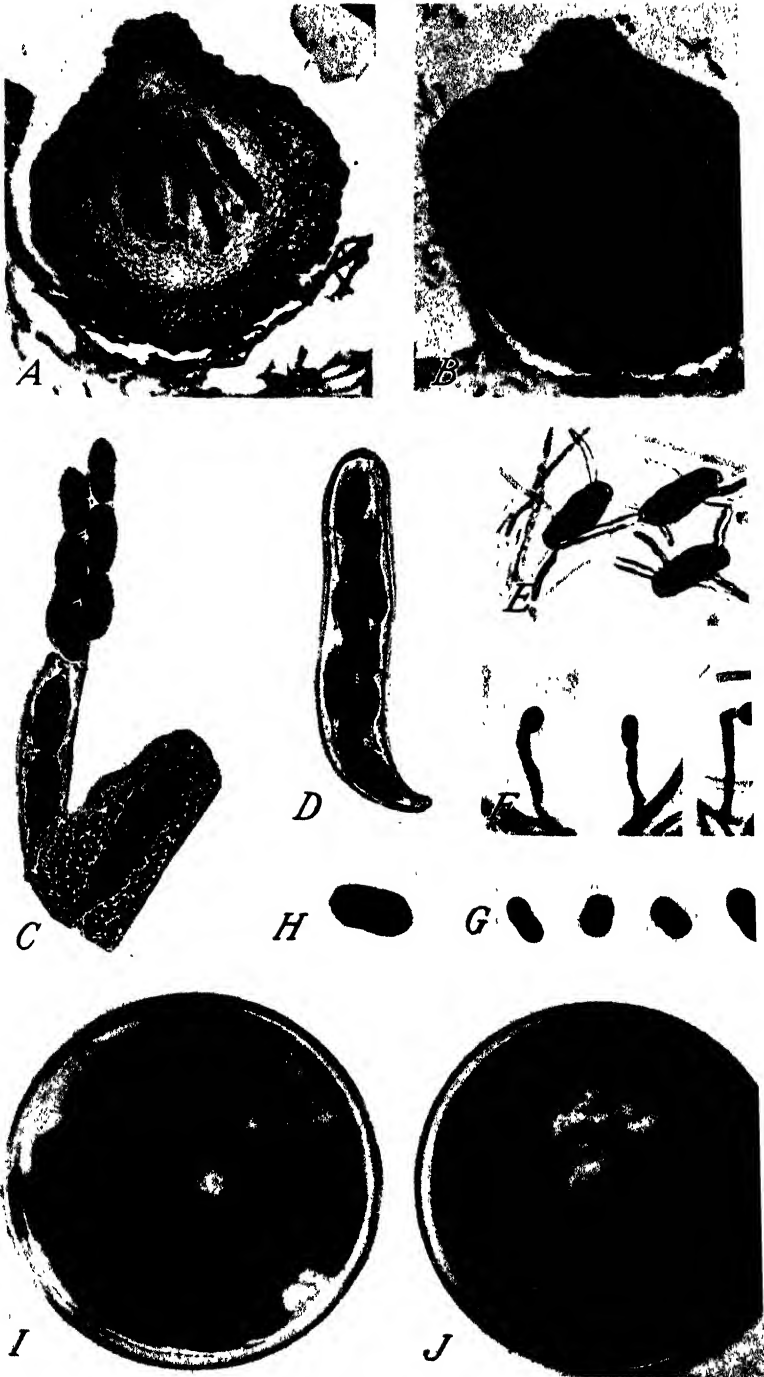
Ripe tomatoes inoculated with *Pleospora* and held at 65° F. develop lesions almost one-half an inch in diameter in 1 week and a little less than five-eighths of an inch in diameter in 10 days. At 70° decayed areas one-half inch in diameter are developed in a week, and these lesions are approximately five-eighths of an inch in diameter within 10 days. Above 70° the rate of enlargement of lesions diminishes rather rapidly. At 75° lesions develop only one-fourth of an inch in diameter in a week, and within 10 days most spots do not reach a diameter greater than five-sixteenths of an inch. Only slight development of decay can be detected after 10 days in fruits held at 80°.

Because of variations in the manner of growth of *Pleospora* in inoculated tomatoes and unavoidable variations in the maturity of the fruits, the size of the lesions developed does not always indicate accurately the effects of the different temperatures upon the fungus. In some instances the fungus grew extensively inside the seed cavity while the external lesions remained relatively small. In making final readings on inoculated tomatoes the lesions were cut and cognizance was taken of the development in the interior of the fruit. For the direct effect of temperature changes upon the growth rate of the fungus reference should be made to figure 1, which shows the diameters of the fungus colonies that developed on the flat surface of potato-dextrose agar in Petri dishes held at the specified temperatures. The growth on artificial media does not correspond exactly with that made in the tomato fruit, but it is interesting to note that the minimum, optimum, and maximum temperatures for the growth of the organism



1. Mature-green tomato showing the early stages of pleospora rot about the stem scar; B, mature-green tomato showing two V-shaped early stages of decay at the edge of the stem scar, and an advanced stage with gray surface mycelium, C, mature-green tomato showing the greenish-brown decay and well-developed grayish-brown mycelium of the *Macrosporium* stage characteristic of tomatoes held on track or in the moist atmosphere of ripening rooms, D, section through stem end of mature-green tomato showing well-developed greenish-brown to brown decay and slight discoloration of the vascular elements underneath the stem, E, advanced stage of decay of ripe tomato showing mature black perithecia.

Pleospora Rot of Tomatoes



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

agree rather closely with the results obtained in the inoculation experiments.

THE CAUSAL ORGANISM

IDENTITY

A check of the measurements of the *Pleospora* isolated from California tomatoes indicates that it is identical with *Pleospora lycopersici* É. and É. Marchal (5), which was described in 1921 as a new fungus causing a fruit rot of tomatoes in Belgium. The conidial stage, determined as *Macrosporium sarcinaeforme* Cav.,³ was also observed at that time, but no description was given of the type of lesions produced.

The measurements for the California strain of *Pleospora lycopersici* as developed on potato-dextrose agar are as follows: Perithecia, $265\mu-375\mu \times 325\mu-550\mu$ average $325\mu \times 425\mu$; asci, $22.5\mu-32.5\mu \times 125.5\mu-192.5\mu$, average $28.2\mu \times 167.0\mu$; ascospores, $12.5\mu-19.0\mu \times 30.0\mu-41.0\mu$, average $15.2\mu \times 34.4\mu$; conidia, $11.0\mu-15.0\mu \times 20.0\mu-36.5\mu$, average $13.5\mu \times 26.0\mu$.

Except for a previous report by the writer (6), apparently no mention has been made of the occurrence of this fungus in the United States.

DEVELOPMENT

When the perithecia become moist or are placed in a drop of water the asci exude through the ostiole. As they emerge they absorb water very rapidly and by breaking through a thin place in the apex of the outer wall (pl. 2, *D*) the inner flexible wall elongates until the ascus is about twice its original length (pl. 2, *C*). The thicker, somewhat gelatinous inner wall continues to absorb water until the internal pressure becomes so great that it breaks. This wall usually ruptures, either at the tip of the ascus or just above the ring (pl. 2, *C*) made by the tip of the outer wall after it contracts towards the base of the ascus, as described by Atanasoff (1) for *Pleospora herbarum* (Pers.) Rabh. In the second instance the ascospores are expelled laterally, while in the first the spores are ejected singly through the small orifice at the apex much after the manner of shooting Roman candles. Hodgetts (3) describes a similar method of spore discharge in *Leptosphaeria acuta* (Moug.) Karst. The ascospores and conidia of *P. lycopersici* germinate readily in water and in tomato juice. In water at room temperature the germ tubes of ascospores develop within 3 hours to the extent shown in plate 2, *E*.

The drawings by É. and Ém. Marchal (5) show the conidia to be smooth-walled. The episporangia of the conidia belonging to the *Pleospora* isolated from California tomatoes are minutely echinulate. In some stages of development the conidia appear smooth, but characteristically the episporangium is definitely echinulate (pl. 2, *F*, *G*, *H*). This character, together with the sarcinaeform type of spore, would place the conidial stage in the genus *Thyrosopora* as established by Tehon and Daniels (8), in which *Thyrosopora sarcinaeforme* (Cav.) comb. nov. is made the type species.

EXPLANATORY LEGEND FOR PLATE 2

A, Longitudinal section through a mature perithecium of *Pleospora lycopersici*. *B*, Longitudinal section through a perithecium showing some stages in the development of asci and spores. *C*, Ruptured ascus showing six spores in the gelatinous matrix of the inner wall and two spores remaining in the foot of the ascus. The ring made by the broken outer wall, which contracts toward the base of the ascus, is shown just below the group of six spores. *D*, Mature ascus before absorption of water and elongation by rupture of outer wall. The small white V-shaped notch in the tip of the ascus marks the thin place in the wall at which rupture occurs. *E*, Ascospores germinated in water. *F*, Conidiophores and conidia showing three developmental stages. *G*, Four mature conidia. *H*, Mature conidium greatly enlarged to show echinulation of the spore wall. *I*, Plate culture of *P. lycopersici* showing white saltant sectors, which produce perithecia but not conidia. *J*, Plate culture of *P. lycopersici* showing the rapidly developed gray mycelium of the *Macrosporium* stage in contrast with the white to light-gray slower growing mycelium of the *Pleospora* stage.

CULTURE STUDIES

DEVELOPMENT OF COLONIES

Cultures grown from single ascospores on potato-dextrose agar usually produce conidia (pl. 2, *F*, *G*) within a week, and shortly thereafter numerous perithecia begin to form (pl. 2, *I*). Mature ascospores are not found within the perithecia (pl. 2, *A*, *B*) until the cultures are 2 to 3 weeks old. Colonies grown from single conidia develop in the manner described above, both conidia and ascospores being formed in the usual length of time required for plantings made from mycelium and ascospores.

CULTURAL CHARACTERS

This fungus, when grown on potato-dextrose agar slants in test tubes, can be separated readily from ordinary *Alternaria* and *Macrosporium* cultures by the rose-colored crescent formed at the top of

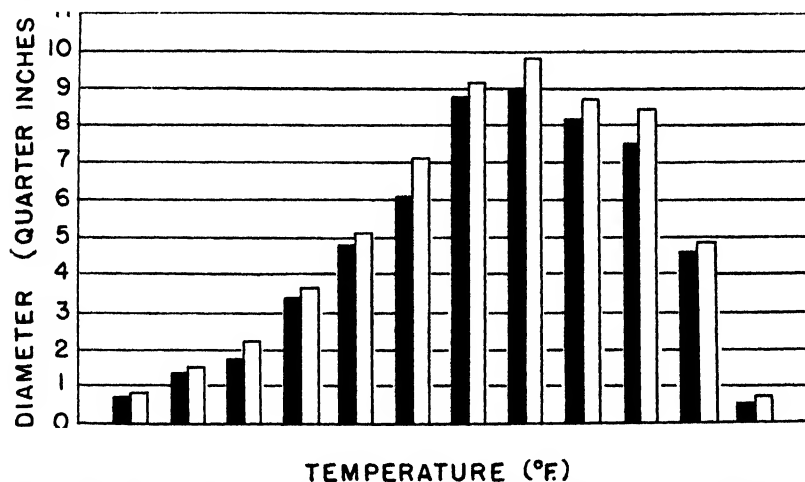


FIGURE 1.—Average diameters of colonies of *Pleospora* developed in 7 days on potato-dextrose agar pH 4.7 (black bars) and pH 6.01 (light bars) at specified temperatures

the slant. This colored crescent is 2 to 4 mm wide, and the pigment does not diffuse into the agar as is characteristic of the chromogenic *Alternarias*. Occasionally certain strains grown in plate cultures at high temperature develop a slight yellowish diffusible pigment, but this is not common for the organism, whereas the rose crescent is characteristic. So far, it has been the writer's experience that all *Macrosporium* cultures that when isolated from tomatoes and other hosts developed this rose crescent have produced a *Pleospora* stage.

Cultures of *Pleospora lycopersici* developed from single spores have a marked tendency to produce saltant sectors such as that shown in plate 2, *I*. Some saltants, like the white V-shaped areas, develop rudimentary perithecia which seldom produce well-formed asci and spores. The remaining dark-gray, more rapidly growing part of the colony bears conidia and few perithecia. In plate 2, *J*, is shown a white cottony sterile growth in the center of the colony and a well-developed gray *Macrosporium* stage bearing conidia in great abundance.

EFFECT OF ACIDITY AND TEMPERATURE ON GROWTH RATE

In figure 1, which shows graphically the diameters of colonies of *Pleospora* that developed in 7 days at different temperatures, data are given on the effects of acidity upon the growth rate of the fungus on an artificial medium. In all experiments of this kind a potato-dextrose agar was used. For direct comparison studies a large amount of this medium was made and then divided into two lots; one lot was titrated to pH 6.01 and the other to pH 4.7. These acidities were arrived at by testing numerous mature-green tomatoes and tomatoes that were ripe enough to be in prime condition for salads. The average pH value of the mature-green fruits was 4.7, whereas that of the ripe tomatoes was 6.01.

The height of the black columns in figure 1 represents the weekly growth in diameter of the fungus culture on potato-dextrose agar of the acidity of mature-green tomatoes (pH 4.7); the light columns indicate the corresponding growth made on agar of the acidity of ripe tomatoes (pH 6.01). In this figure it is readily seen that at all temperatures at which appreciable development was made the fungus grew more rapidly on the less acid medium. This is in complete accord with transit and market observations made on naturally infected and on artificially infected tomatoes, all of which showed more rapid decay in the ripening fruits. It should be noted, however, that the difference in the amount of decay made on green and ripe fruits is proportionately much greater than the difference between the size of the colonies developed on agar at pH 4.7 and 6.01 at corresponding temperatures. A probable explanation of this difference in the rate of development of the organism is that changes other than those in acidity, made during the ripening of the fruit, are also important in influencing the growth rate and consequently the amount of decay induced by this organism.

The minimum temperature for the development of *Pleospora lycopersici* on potato-dextrose agar (pH 4.7 and 6.01) is approximately 35° F., and the maximum is 90°. Slight growth is made at these temperatures for the first 2 or 3 days and then all development ceases, although the organism is not killed at either of these extremes. The optimum temperature for the development of this fungus is 70°. As indicated in the graph, very poor growth is made at all temperatures up to 55° and above 85°, while at the temperatures at which tomatoes are shipped and ripened (60° to 80°) growth is favored. Change in the growth rate could be detected whenever the fungus showed a marked tendency toward developing either the conidial stage or the perfect stage to the exclusion or near exclusion of the other. The conidial stage, *Macrosporium sarcinaeforme*, always grew faster at higher temperatures, and its optimum was about 5° higher (75°) than that for the *Pleospora* stage. The colony illustrated in plate 2, J, which was developed from a single central planting, shows the variability in growth rate of the different stages of this organism.

SUMMARY AND CONCLUSIONS

Pleospora rot of tomatoes was first recognized in the United States in a car of California tomatoes received on the Chicago market in November 1919. Pure cultures of the causal organism were obtained at that time, and since then numerous other isolations of the fungus

have been obtained. Pleospora rot has been found only in California tomatoes marketed during November and December and in Mexican stock shipped in January.

The disease induced by *Pleospora* has become increasingly important during the past 3 years. Losses of as high as 50 to 90 percent of the tomatoes in some cars have been reported. Infections may take place in the tomatoes while still in the field or during harvesting and packing. Decay develops slowly during the first 3 or 4 days in transit while the fruits are green, but as they ripen decay progresses more rapidly. Spots varying from one-fourth to three-fourths of an inch in diameter may develop during transit. Greenish-brown to brown moderately firm decayed areas involving one-fourth of a fruit have been developed in ripening tomatoes within 10 days after shipment. The *Macrosporium* stage is evident on the fruits at the time of arrival on the markets, and in most lesions one-half an inch in diameter the *Pleospora* stage is also evident.

Mature-green tomatoes inoculated with *Pleospora lycopersici* and held at various temperatures show little or no decay below 45° F., or above 80°. The greatest development of decay takes place at 65° to 70°. Decay developed more rapidly in inoculated ripe tomatoes at all temperatures, but the minimum, optimum, and maximum temperatures were about the same as for green fruits.

The fungus isolated from California tomatoes has been found to be identical with *Pleospora lycopersici*, which was described in 1921 by É. and Ém. Marchal as a new fungus causing decay of tomatoes in Belgium. The conidial stage, *Macrosporium sarcinaeforme* Cav., was reported by them, and it has been found constantly associated with the *Pleospora* stage in California tomatoes. Single-spore cultures made from either ascospores or conidia give rise to both the *Pleospora* and *Macrosporium* stages of the fungus.

On potato-dextrose agar (pH 4.7 and 6.01) the minimum temperature for growth of the fungus was 35° F., the optimum 70°, and the maximum 90°. In cultures having a distinct tendency toward producing the *Macrosporium* stage the growth rate was more rapid than in those cultures in which the *Pleospora* phase was dominant. The optimum temperature for the development of the *Macrosporium* stage alone was about 75°. At all temperatures at which appreciable development of the fungus was made, the growth rate was more rapid on the agar having a pH value of 6.01 (the average acidity of ripe tomatoes) than on one having a pH value of 4.7 (the average acidity of mature-green tomatoes). This harmonizes with the fact that most rapid and serious decay induced by *Pleospora lycopersici* takes place on the market in tomatoes that are in the turning and ripe stages.

LITERATURE CITED

- (1) ATANASOFF, D.
1919. A NOVEL METHOD OF ASCOSPORE DISCHARGE. *Mycologia* 11: 125-128, illus.
- (2) DOUGLAS, B.
1922. A NEW ALTERNARIA SPOT OF TOMATOES IN CALIFORNIA. *Phytopathology* 12: 146-148, illus.
- (3) HODGETTS, W. J.
1917. ON THE FORCIBLE DISCHARGE OF SPORES OF LEPTOSPHERIA ACUTA. *New Phytol.* 16: 139-146, illus.
- (4) MCWHORTER, F. P.
1927. THE EARLY-BLIGHT DISEASES OF TOMATOES. *Va. Truck Exp. Sta. Bull.* 59, pp. [547]-566, illus.
- (5) MARCHAL, ÉL., and MARCHAL, ÉM.
1921. CONTRIBUTION À L'ÉTUDE DES CHAMPIGNONS FRUCTICOLES DE BELGIQUE. *Bull. Soc. Roy. Bot. Belg.* 54: [109]-139, illus.
- (6) RAMSEY, G. B.
1934. PLEOSPORA LYCOPERSICI E. AND E. MARCH., A TOMATO PATHOGEN IN THE UNITED STATES. *Science (n. s.)* 79: 294.
- (7) ——— and LINK, G. K. K.
1932. MARKET DISEASES OF FRUITS AND VEGETABLES: TOMATOES, PEPPERS, EGGPLANTS. *U. S. Dept. Agr. Misc. Pub.* 121, 44 pp., illus.
- (8) SAMUEL, G.
1932. MACROSPORIUM SOLANI ON TOMATO FRUIT. *Phytopathology* 22: 613-614, illus.
- (9) TEHON, L. R., and DANIELS, E.
1925. A NOTE ON THE BROWN LEAF-SPOT OF ALFALFA. *Phytopathology* 15: 714-719, illus.

A CHEMICAL INVESTIGATION OF THE FERMENTATIONS OCCURRING IN THE PROCESS OF POI MANUFACTURE¹

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INTRODUCTION

In a bulletin of the Hawaii Agricultural Experiment Station (2)² Allen and Allen discussed, from the bacteriological standpoint, the fermentations occurring in the preparation of poi. They concluded that the fermentation of poi might be divided into two phases, the first, covering 5 or 6 days, in which bacteria of a high acid-producing type predominated; and the second, extending from the third to the sixth day, in which a flora of yeasts, mycoderms, and oidia was increasingly prevalent. This paper reports the results of an attempt to identify chemically the products of bacterial action shown by Allen and Allen to occur in poi at different stages of fermentation.

So far as the specific products of fermentation are concerned, the study resulted in qualitative determinations only. Attempts to secure quantitative data led to the conclusion that the physical nature of poi, variations in samples, exceedingly small quantities of fermentation products, and the effect of even very slight variations in conditions upon such small quantities rendered quantitative determinations practically impossible. The quantities could only be roughly indicated.

Preliminary to the identification of the specific products of fermentation, quantitative determinations were made of starch, reducing sugars, volatile acids, and nonvolatile acids in fresh poi³ and in poi at various stages of fermentation. These data proved of value in predicting the products of the chemical and bacteriological changes. Quantities of fresh poi were obtained as needed from a poi factory in Honolulu. Attempts to use frozen poi were abandoned early in the work because it was found difficult to secure the uniformity of samples necessary for analytical purposes.

ANALYSIS OF FRESH POI

Methods used in the analysis of fresh poi for moisture, starch, pentosans, reducing sugars, sucrose, proteins, fat, crude fiber, ash, phosphorus, and calcium were standard methods taken, in the main, from Allen (1, *v. 1*), Official and Tentative Methods of Analysis (3), and Browne (4). Reducing sugars were determined by McAllep's

¹ Received for publication Feb. 20, 1934; issued August 1935.

² Reference is made by number (italics) to Literature Cited, p. 49.

³ The manufacture of poi is described by Allen and Allen (2). After taro has been cooked, ground, and mixed with water, the product is known as fresh poi. Incubation and fermentation follow.

methylene blue method (7). The results, which are averages of triplicate determinations, are given in the following tabulation:

	Percent		Percent
Moisture.....	69.3	Fat.....	0.07
Starch (takadiastase).....	27.0	Crude fiber.....	.39
Pentosans.....	1.3	Ash.....	.46
Reducing sugars.....	.5	Phosphorus.....	.057
Sucrose.....	.03	Calcium.....	.018
Proteins.....	.31		

These data account for approximately 99.5 percent of the weight of poi used for analysis and, considering unavoidable variations in samples, the starch and reducing sugars found check well with those recorded in table 1 for 0-hour poi.

DETERMINATION OF STARCH AND REDUCING SUGARS PRESENT IN POI AT THE VARIOUS STAGES OF ITS FERMENTATION

Starch and reducing sugars were first determined together by acid hydrolysis of the starch. Then reducing sugars alone were determined by the colorimetric blood sugar method (6). The percentage of starch was obtained by difference. The chief difficulty encountered in this analysis lay in the mechanical separation of reducing sugars from starch. Centrifuging poi and water mixtures for 30 minutes at 1,500 revolutions per minute was substituted for filtering. The variations in the amounts of reducing sugars and starch present in poi during fermentation from the stage of fresh poi, 0 hour, to sour poi, 8 days, are shown in table 1.

TABLE 1.—*Reducing sugars and starch in poi at various stages of fermentation*

Age of poi at time analyzed	Reducing sugars	Starch	Age of poi at time analyzed	Reducing sugars	Starch	Age of poi at time analyzed	Reducing sugars	Starch
	Per-cent	Per-cent		Per-cent	Per-cent		Per-cent	Per-cent
0 hour.....	0.53	25.6	24 hours.....	0.10	26.0	5 days.....	0.12	23.4
6 hours.....	.48	25.8	2 days.....	.11	25.8	6 days.....	.15	23.2
12 hours.....	.14	26.0	3 days.....	.14	24.5	7 days.....	.18	23.2
18 hours.....	.10	25.8	4 days.....	.12	23.9	8 days.....	.15	22.2

Allen and Allen (2) state, from bacteriological evidence only, that there is little doubt that the fermentation of poi is a complex one and that the immediate evidence of fermentation, such as the evolution of carbon dioxide and an increase in acidity, indicate that carbohydrates are among the first substances to be attacked. The data in table 1 show a rapid decrease of sugars early in the fermentation. The fair degree of constancy of the percentage of reducing sugars from 12 hours to 8 days is consistent with the continuous diminution in starch content. The fairly constant values found for reducing sugars after the first 12 hours may be accounted for by a counterbalancing due to starch hydrolysis, or the constant values may indicate that all reducing sugars had been fermented and that the values found result from nonfermentable reducing substances present in the poi.

The decrease in starch content shown in table 1 was predicted by Allen and Allen (2) and is consistent with the anticipated behavior of the micro-organisms found by them. They state that there is reason to believe that during the period of acid production considerable starch is hydrolysed and thus converted into available energy. However, Miller (8), who studied the microbial fermentation of poi, failed to find any appreciable decrease in starch content. Allen and Allen (2), in commenting on Miller's data, state that since a pH value was not given for the fresh poi, the sample very probably was comparatively acid at the time of starch analysis and hydrolysis had already occurred.

DETERMINATION OF VOLATILE AND NONVOLATILE ACIDS PRESENT IN POI AT THE VARIOUS STAGES OF ITS FERMENTATION

The methods described in Official and Tentative Methods of Analysis (3) were used to determine volatile and nonvolatile acids. Fifty-gram samples of poi of the ages shown in table 2 were used. Each sample was mixed with 100 cc. of water and centrifuged for 5 minutes at 1,500 revolutions per minute. The supernatant liquid was decanted, additional water placed in the centrifuge tubes, and the centrifuging and decanting repeated until 500 cc. of a water extract of the poi sample was obtained. The extract was distilled by ordinary distillation with periodic checking of the distillate by titration and the addition of water to the distilling flask, as described in Official and Tentative Methods of Analysis (3). Volatile and nonvolatile acids were determined by titration with 0.2 normal sodium hydroxide solution. The results are given in table 2.

TABLE 2.—Volatile and nonvolatile acids expressed as milligrams of hydrogen in 50-g samples

Age of poi at time analyzed	Volatile acids	Non-volatile acids	Age of poi at time analyzed	Volatile acids	Non-volatile acids	Age of poi at time analyzed	Volatile acids	Non-volatile acids
0 hour.....	1.00	0.93	1 day.....	1.60	1.40	7 days.....	3.56	4.41
2 hours.....	.87	.99	3 days.....	3.15	2.07	10 days.....	5.75	4.36

The micro-organisms found in Allens' bacteriological study to be present in poi are capable of inducing chemical changes which result in the formation of both volatile acids such as formic, acetic, propionic, and butyric, and nonvolatile acids such as lactic, oxalic, citric, malic, tartaric, and succinic. Those micro-organisms responsible for the volatile acids are known to be more active in the later stages of fermentation of poi than in the earlier stages.

IDENTIFICATION OF SPECIFIC SUBSTANCES PRESENT IN POI AT VARIOUS STAGES OF ITS FERMENTATION

The results of the complete analysis of poi, the study of reducing sugars and starch, the determination of volatile and nonvolatile acids, and the identification of micro-organisms through the bacteriological investigation made possible some definite predictions as to products resulting from the chemical changes induced by the micro-

organisms. Allen and Allen (2) state that the acid-producing bacteria of the *Streptococcus* and *Lactobacillus* species, the principal agents of fermentation, are able to produce not only large quantities of lactic acid but also moderate quantities of acetic, propionic, succinic, and formic acids, in addition to traces of acetone and alcohol. The species of yeasts, mycoderms, and oidia, present in much smaller numbers, could not account for the major chemical changes but could cause the formation of alcohol, carbon dioxide, and some acetic acid.

In the chemical study search was made for lactic, acetic, formic, propionic, butyric, malic, tartaric, succinic, citric, and oxalic acids, carbon dioxide, alcohol, acetone, and aldehyde at the stages from 0 hour to 10 days. Table 3 summarizes this study and is followed by a discussion of results.

TABLE 3.—Results of experimentation to determine specific substances resulting from chemical changes induced by micro-organisms at various stages of poi fermentation¹

Substance found	0 hour	2 hours	1 day	3 days	5 days	7 days	10 days
Lactic acid.....	++	++	+++	+++	+++	+++	+++
Acetic acid.....	0	++	++	++	+++	+++	+++
Formic acid.....	0	++	++	++	++	++	++
Alcohol.....	0	+	+	+	+	+	+
Acetaldehyde.....	0	0	+	+	+	+	+
Carbon dioxide.....	+++	+++	+++	+++	+++	+++	+++

¹ 0 indicates the absence of the indicated substance, + that traces were found, ++ that the substance was present in small amounts, and +++ that appreciable quantities were present.

The treatment of poi samples and the methods used in testing for the various substances referred to in table 3 were briefly as follows: Alcoholic extracts (1, v. 1) of lactic, acetic, and formic acids, were obtained by triturating 100-g samples with 75 cc of 95-percent alcohol. The alcoholic extracts were filtered, diluted, made alkaline with excess of sodium carbonate, concentrated to small volumes, clarified with a decolorizing carbon, neutralized with hydrochloric acid, and boiled to remove carbon dioxide. These solutions were subjected to qualitative tests (1, v. 1; 5). To detect lactic, formic, and acetic acids, qualitative tests (1, v. 1; 5) were also applied to properly prepared samples, obtained from the distillation of volatile acids by the Dyer method (5).

The guaiacol, iodoform, aldehyde, and ferric chloride tests were used for lactic acid. Also, calcium lactate crystals were prepared from a sample of 900 g of fresh poi and identified by qualitative tests and by microscopic comparison with crystals of U. S. P. calcium lactate. For acetic acid, the concentrated sulphuric acid, ethyl acetate, and ferric chloride tests were applied. The mercuric chloride, silver nitrate, and ferric chloride tests were used for formic acid (1, v. 1; 5).

Tests for alcohol (1, v. 1; 9) and for acetaldehyde (1, v. 1; 9) were applied to samples of the distillates obtained in the determination of volatile acids and also to samples prepared as follows: One-hundred-gram samples of poi, at the fermentation stages indicated in table 3, were distilled with 150 cc of water, made slightly alkaline with 6 normal sodium hydroxide, and distilled over a water bath at a temperature of 50° C. and under pressure of 100 mm until 100 cc of distillate were

obtained. For alcohol, the ethyl acetate, ethyl benzoate, iodoform, and alkaline potassium permanganate tests were used, and for acetaldehyde, the Fehling, ammoniacal silver nitrate, aldehyde resin, and Nessler tests were applied.

Propionic acid, butyric acid, higher fatty acids (5), malic acid, tartaric acid, succinic acid, citric acid, oxalic acid (1, *v. 1*), and acetone (1, *v. 1*) were absent at all stages of fermentation. Tests for these substances were made both on alcoholic extracts of poi and on residues remaining from the distillation of volatile acids.

While it is known that a butyric ferment is present in poi, it was proved that lactic acid is not converted to butyric acid under ordinary conditions of fermentation either in the early stages of fermentation or in poi which has fermented as long as 15 days. However, butyric acid was isolated and identified (1, *v. 1*; 5) from a mixture of poi and calcium carbonate which had been allowed to stand for 15 days. No butyric acid was obtainable from poi in earlier stages of fermentation even in the presence of calcium carbonate. The well-known unpleasant odor and taste of very old poi may be accounted for by the formation of butyric acid as the acidity diminishes upon long standing.

SUMMARY AND CONCLUSIONS

The chemical investigation of the fermentations occurring in poi manufacture included a complete analysis of poi, the determination of reducing sugars, starches, volatile acids, and nonvolatile acids present at various stages of fermentation, and the identification of the products of fermentation. The analysis showed poi to be a starchy food consisting of about 69 percent water, 27 percent starch, and relatively small amounts of other substances. Reducing sugars decreased rapidly in the early stages of fermentation and remained fairly constant in amount from the stage of 12 hours to 8 days. Starches decreased from the second to the eighth day of fermentation.

The fermentation products identified were lactic acid, acetic acid, formic acid, alcohol, acetaldehyde, and carbon dioxide. These are consistent with the predictions made from the bacteriological study of the micro-organisms described by Allen and Allen (2). The results of the investigation show that the fermentations occurring in poi manufacture are due primarily to the action of micro-organisms on carbohydrates.

LITERATURE CITED

- (1) ALLEN, A. H.
1923-33. ALLEN'S COMMERCIAL ORGANIC ANALYSIS . . . Ed. 5, rev. and in part rewritten . . . 10 v., illus. Philadelphia.
- (2) ALLEN, O. N., and ALLEN, E. K.
1933. THE MANUFACTURE OF POI FROM TARO IN HAWAII: WITH SPECIAL EMPHASIS UPON ITS FERMENTATION. Hawaii Agr. Expt. Sta. Bull. 70, 32 pp., illus.
- (3) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. Ed. 3, 593 pp., illus. Washington, D. C.
- (4) BROWNE, C. A.
1912. A HANDBOOK OF SUGAR ANALYSIS; A PRACTICAL AND DESCRIPTIVE TREATISE FOR USE IN RESEARCH, TECHNICAL AND CONTROL LABORATORIES. 787 pp., illus. New York.

- (5) DYER, D. C.
1917. A NEW METHOD OF STEAM DISTILLATION FOR THE DETERMINATION OF THE VOLATILE FATTY ACIDS, INCLUDING A SERIES OF COLORIMETRIC QUALITATIVE REACTIONS FOR THEIR IDENTIFICATION. *Jour. Biol. Chem.* 28: 445-473, illus.
- (6) FOLIN, O.
1922. LABORATORY MANUAL OF BIOLOGICAL CHEMISTRY, WITH SUPPLEMENT. 300 pp., illus. New York and London.
- (7) McALLEP, W. R.
1926. METHYLENE BLUE METHOD FOR GLUCOSE DETERMINATIONS. *Hawaii. Planters' Rec.* 30: 461-465.
- (8) MILLER, C. D.
1927. FOOD VALUES OF POI, TARO, AND LIMU. *Bernice P. Bishop Mus. Bull.* 37, 25 pp., illus.
- (9) NORRIS, J. F.
1922. THE PRINCIPLES OF ORGANIC CHEMISTRY. 631 pp., illus. New York.

RESULTS OF FEEDING SPROUTED OATS TO CORRECT STERILITY IN CATTLE AND SWINE¹

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INTRODUCTION

The idea that sprouted oats might have value in restoring breeding power to cows temporarily sterile due to diet deficiencies probably had its origin in the work of Evans and Bishop² and others. These writers, working with rats, demonstrated the existence of a hitherto unrecognized vitamin which was later called vitamin E.

Preliminary experiments on the feeding of sprouted oats to temporarily sterile cows were initiated as early as 1923, and more elaborate experiments were started in 1925 at the Beltsville dairy farm of the Bureau of Dairy Industry of the United States Department of Agriculture.³ This work was continued, and in 1930 the results of feeding sprouted oats to cows which were temporarily sterile and in which no pathological conditions could be found were given by Graves.⁴ Miller and Graves⁵ in reporting on the health records of the Beltsville herd give details on the methods of feeding sprouted oats and report that 57 females were directly benefited and 31 were not benefited, as judged by the number of services required to produce conception. In 27 cases, the cows conceived to a service just previous to the start of the oat feeding, and it is not definitely known whether such feeding was beneficial. There was no evidence that sprouted oats were helpful to the cows showing pathological changes in the genital organs.

Moore⁶ of the Mississippi Agricultural Experiment Station fed sprouted oats to Jersey and Ayrshire heifers which had failed to breed. In several cases such oat feeding was helpful, but in others the results were negative.

EXPERIMENTS WITH CATTLE

EARLY OBSERVATIONS AT THE HAWAII STATION

The feeding of sprouted oats to heifers and cows that exhibited an abnormal breeding behavior was started in January 1927. These included animals which seemingly did not have the normal oestrus periods or, if the periods occurred normally, did not conceive when bred. Oats were germinated in trays and fed at the rate of 2 pounds (dry weight before germinating) per day until such time as the cows were thought to be pregnant. This plan was continued for about 3½ years.

¹ Received for publication Mar. 19, 1935; issued August 1935.

² EVANS, H. M., and BISHOP, K. S. ON THE EXISTENCE OF A HITHERTO UNRECOGNIZED DIETARY FACTOR ESSENTIAL FOR REPRODUCTION. *Science* (n. s.) 56: 650-651. 1922.

³ GRAVES, R. R. DAIRY CATTLE BREEDING INVESTIGATIONS. U. S. Dept. Agr., Bur. Dairy Indus. Ann. Rept. of Chief, 1926, p. 5. 1926.

⁴ ———. DIVISION OF BREEDING, FEEDING, AND MANAGEMENT INVESTIGATIONS. U. S. Dept. Agr., Bur. Dairy Indus. Ann. Rept. of Chief, 1930, p. 17. 1930.

⁵ MILLER, F. W., and GRAVES, R. R. REPRODUCTION AND HEALTH RECORDS OF THE BELTSVILLE HERD OF THE BUREAU OF DAIRY INDUSTRY. U. S. Dept. Agr. Tech. Bull. 321: 15-17. 1932.

⁶ MOORE, J. S. FEEDING SPROUTED OATS TO OVERCOME DIFFICULT BREEDING. *Miss. Agr. Expt. Sta. Ann. Rept.* (1926-27) 40: 23. 1927.

Six of these oat-fed cows were animals that had failed to show any definite signs of being in heat after an average of 192 days after calving. Four of them exhibited heat periods and were successfully bred after an average of 13 days of oat feeding. A fifth cow was successfully bred in her second oestrus, which occurred 39 days after a 17-day oat-feeding period had been discontinued. The sixth cow was fed sprouted oats for 110 days, during which time she had two heat periods and was bred, but neither of these 2 services or the 6 that followed over a period of 1 year resulted in pregnancy.

One other cow had had 5 services, none of which resulted in pregnancy. This cow was then fed sprouted oats for 171 days, during which time there were 4 more services, none of which resulted in conception.

EXPERIMENTS WITH FEEDING OATS

As the early observations, while lacking controls, were in general rather favorable to sprouted oats as a feed, a more definite experiment was planned which began November 1, 1930. For this experiment, the following classes of cows were considered as having abnormal breeding behavior:

Class 1, heifers 24 months of age or older that seemingly show no heat periods. Such heifers were fed sprouted oats from the time they were 24 months old till 70 days after the last bull service.

Class 2, cows that had not come in heat within 4 months after calving. Such cows were fed sprouted oats after this 4-month period till 70 days after the last bull service.

Class 3, cows and heifers that had been bred once but had come in heat again. Such cows were fed sprouted oats till 70 days after the last service by the bull.

Class 4, the cows that had been the control groups in classes 1, 2, and 3 during the earlier part of the experiment and were still nonpregnant.

This plan included all animals in the herd except such as might be on some special test which would confuse the results. The first animal which fitted into one of the classes was fed sprouted oats as indicated; the second cow was not fed oats but was considered as a control animal; the third cow was fed oats; the fourth was not, etc. All the experimental animals received ample green feed daily.

Oats were germinated in trays and fed when the sprouts were from one-half to 2 inches long, at the rate of 2 pounds of oats (dry basis) per cow per day. For the most part, the cows ate the sprouted oats fairly well.

The tables show the results of feeding oats in the various classes. The reaction of each cow to the agglutination test for abortion is shown in all cases, the first character showing reaction at 1 to 25 dilution, the second at 1 to 50 dilution, and third at 1 to 100 dilution.

Table 1 gives the record of the cows in class 1.

TABLE 1.—Record of individual cows in class 1

Group	Cow no.	Agglutination test ¹	Period from 24 months of age to service that resulted in pregnancy or last service previous to sale	Period oats were fed		Bull services		Calf produced
				Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
Fed oats.....	{ 103G 112H	{ — — — P — — —	<i>Days</i> 460 2	<i>Days</i> 113 2	<i>Days</i> 0 69	<i>Number</i> 0 0	<i>Number</i> 5 1	Yes. Yes.
Control ²	{ 106H 121G	{ + — — — — —	91 343	----- -----	----- -----	4 1	----- -----	Yes. No.

¹ Capital P means partial, plus sign (+) positive, minus sign (—) negative.² Oats not fed.

Pregnancy did not occur in cow 103G till 353 days after sprouted-oats feeding had been stopped. Pregnancy occurred in cow 112H only 2 days after the feeding of oats was started. It is doubtful whether oats feeding was a determining factor in either case.

The data for the individual cows in class 2 that were continued in the herd long enough to get final calving data are shown in table 2.

TABLE 2.—Record of individual cows in class 2

Group	Cow no.	Agglutination test ¹	Date of last previous parturition	Period from calving to first observed heat period	Period from calving to service that resulted in pregnancy or last service previous to sale	Period oats were fed		Bull services		Calf produced
						Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
				<i>Days</i>	<i>Days</i>	<i>Days</i>	<i>Days</i>	<i>Number</i>	<i>Number</i>	
Fed oats...	97H	P — —	Aug. 11, 1930	191	369	227	0	0	4	Yes.
	100H	— — —	Nov. 7, 1930	165	299	91	0	0	2	Yes.
	86G	— — —	July 12, 1931	240	240	111	70	0	1	Yes.
	92H	— — —	July 31, 1931	141	426	99	0	0	4	Yes.
	49H	+ + +	Nov. 8, 1931	159	159	38	69	0	1	Yes.
	101G	— — —	Aug. 29, 1932	156	156	2	70	0	1	Yes.
	72H	— — —	Jan. 9, 1933	140	140	4	70	0	1	Yes.
	65H	— — —	Jan. 20, 1933	130	415	75	0	0	3	Yes.
	108H	— — —	Jan. 24, 1933	165	205	84	70	0	3	Yes.
	91G	+ + +	June 2, 1930	175	479	-----	-----	5	-----	Yes.
	95H	— — —	Aug. 12, 1930	124	430	-----	-----	5	-----	Yes.
	99H	+ — —	Dec. 11, 1930	168	168	-----	-----	1	-----	Yes.
	62H	— — —	May 4, 1931	195	214	-----	-----	2	-----	Yes.
	104H	— — —	July 20, 1931	139	139	-----	-----	1	-----	Yes.
Control ² ...	31H	+ — —	Sept. 12, 1931	133	133	-----	-----	1	-----	Yes.
	72H	+ — —	Oct. 16, 1931	178	178	-----	-----	1	-----	Yes.
	109H	P — —	Aug. 1, 1932	169	169	-----	-----	1	-----	Yes.
	113H	— — —	Aug. 27, 1932	-----	-----	-----	-----	0	-----	No.
	86G	— — —	Dec. 17, 1932	152	152	-----	-----	1	-----	Yes.

¹ Capital P means partial, plus sign (+) positive, minus sign (—) negative.² Oats not fed.

All of the 9 oat-fed cows eventually produced calves; 9 of the 10 cows in the control group also produced calves. The 9 oat-fed cows were fed sprouted oats for an average of 81 days before pregnancy, and in addition 5 of them were fed oats for 70 days each after pregnancy. The plan was to feed all of them sprouted oats for 70 days after pregnancy, but in the case of 4 of the cows the final breeding period, which resulted in pregnancy, did not occur till 70 or more days after the previous service, which was assumed to have resulted in conception.

For the cows which produced calves, an average of 2.2 services were required per conception in both the oat-fed and the control groups.

Two of the cows, 72H and 86G, appear in both the oat-fed and control groups but for different gestation periods. This is possible since this experiment covered a period of nearly 4 years.

For the 9 oat-fed cows an average of 165 days occurred from the time of calving to the first observed heat period and 268 days to the service that finally resulted in pregnancy; for the 9 cows in the control group, the corresponding figures are 159 and 229 days, respectively.

The data for the cows in this class fail to show that feeding sprouted oats was of any value in bringing about conditions favorable to pregnancy.

In class 3, oat feeding was started after the first heat period that occurred after the one when the cow was first bred and continued for 70 days after the last heat period when the cow was bred. Seventy days provides time enough for three normal oestrus periods. If none occurred, it was assumed that the cow was pregnant and oat feeding was stopped. Actually, however, in 11 cases out of 27, heat periods did occur later after none had been observed in the 70-day oat-feeding period. In these cases after oat feeding had been stopped it was not started again, and oats were fed only previous to pregnancy, because the cow was believed to be pregnant when oat feeding was stopped. The results are shown in table 3.

Of 27 oat-fed cows and heifers in class 3, 20, or 74 percent, produced calves; of the 25 control animals, 18, or 72 percent, produced calves. The cows in the oat-fed group were fed oats an average of 115 days each, and for the 20 cows that produced calves, 3.4 services were required per conception. In the control group the 18 cows that produced calves required 4.2 services per conception.

Since a period of about 4 years elapsed during this experiment, a cow might appear twice in the same group or in both groups, for, as previously explained, every other cow exhibiting the same apparent breeding trouble, in the order in which it occurred, was fed oats. Thus, 2 cows appear twice each in the oat-fed group, 3 appear twice each in the control group, and 8 appear in both groups.

Of the oat-fed group, 7 cows were fed oats both before and after pregnancy; 12 were oats fed only before pregnancy and 8 only after pregnancy.

For the 18 oat-fed cows (heifers excluded) that produced calves, an average of 282 days elapsed between the last previous parturition and the service that finally resulted in pregnancy; for the 15 control cows (heifers excluded), an average of 252 days was required.

TABLE 3.—Record of individual cows in class 3

Group	Cow no.	Agglutination test ¹	Date of last previous parturition	Period from calving to service that resulted in pregnancy or last service previous to sale	Period oats were fed		Bull services		Calf produced
					Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
Fed oats-----	51G	- - -	Apr. 21, 1929	Days 758	Days 64	Days 70	7	2	Yes.
	80G	- - -	July 19, 1929	472	70	0	2	0	No.
	64H	P - -	Feb. 9, 1930	272	0	70	4	0	Yes.
	98H	- - -			138	0	4	4	No.
	49H	+ + +	Feb. 12, 1930	325	0	70	2	0	Yes.
	45H	- - -	Mar. 30, 1930	1, 112	449	0	3	12	No.
	74H	+ - -	May 29, 1930	214	19	78	2	1	Yes.
	31H	+ + -	June 10, 1930	185	0	70	2	0	Yes.
	89G	- - -	July 18, 1930	246	0	70	2	0	Yes.
	72H	P - -	Sept. 12, 1930	132	0	115	2	0	Yes.
	108H	- - -			0	70	2	0	Yes.
	96G	- - -	Nov. 27, 1930	263	70	0	2	1	Yes.
	42H	- - -	Jan. 9, 1931	173	20	70	2	1	Yes.
	77G	+ + +	Jan. 30, 1931	301	191	70	2	4	Yes.
	52H	- - -	May 3, 1931	414	70	0	4	0	Yes.
	101G	- - -	July 3, 1931	135	12	71	2	1	Yes.
	60H	- - -	Oct. 16, 1931	220	0	70	2	0	Yes.
	89G	- - -	Jan. 4, 1932	207	0	70	2	0	Yes.
	114G	- - -			69	0	2	1	No.
	102H	- - -	Apr. 25, 1932	258	69	0	2	6	No.
	122H	- - -			70	0	2	1	Yes.
	83H	- - -	July 11, 1932	252	17	70	2	1	Yes.
	111H	- - -	July 29, 1932	471	105	0	2	2	Yes.
	82H	- - -	Sept. 3, 1932	225	87	71	2	3	Yes.
	105H	- - -	Sept. 21, 1932	290	86	0	2	2	Yes.
	85H	- - -	Oct. 14, 1932	535	287	0	2	9	No.
	42H	- - -	Mar. 30, 1933	385	117	0	2	5	No.
	90H	- - -	Dec 15, 1929	392			5		Yes.
	82H	- - -	May 31, 1930	184			3		Yes.
	32G	- - -	June 29, 1930	367			4		No.
	68H	+ + +	June 30, 1930	194			5		Yes.
	94G	- - -	July 9, 1930	288			5		Yes.
	105H	- - -					9		Yes.
	65H	- - -	Sept. 23, 1930	199			4		Yes.
	107G	- - -					4		Yes.
Control ² -----	83H	P - -	Nov. 19, 1930	324			6		Yes.
	59H	+ + +	Nov. 23, 1930	496			9		No.
	67G	+ + +	Dec 26, 1930	199			4		Yes.
	109H	+ - -					3		Yes.
	85H	- - -	Jan. 14, 1931	367			8		Yes.
	88H	- - -	Mar. 10, 1931	112			2		Yes.
	71H	- - -	July 29, 1931	367			2		Yes.
	79H	P - -	Aug. 19, 1931	326			4		Yes.
	74H	P - -	Oct. 1, 1931	202			2		Yes.
	94G	- - -	Feb. 7, 1932	255			4		No.
	117G	- - -					2		No.
	110H	- - -	Feb. 28, 1932	240			2		No.
	51G	- - -	Mar. 7, 1932	311			3		No.
	77G	+ P -	Sept. 2, 1932	154			4		No.
	68H	- - -	Oct. 12, 1932	235			4		Yes.
	112H	- - -	Dec. 17, 1932	123			2		Yes.
	74H	P - -	Jan. 28, 1933	265			3		Yes.

¹ Capital P means partial, plus sign (+) positive, minus sign (-) negative.² Oats not fed.

Examinations for abnormal conditions in the reproductive organs of the cows were made in only a few cases.

Except that the number of services required per conception were slightly lower in the case of the oat-fed cows, there is little else to suggest that feeding oats was helpful to the cows in this class.

During the latter part of the experiment, the cows that had formed the control groups of the first three classes were designated as class 4 and were fed sprouted oats to note their effect on animals that had

failed to conceive normally over a long period. Class 4 included all controls in the dairy that had not had a heat period or had had a reoccurrence of a heat period 7 months or later after calving, and heifers that had not yet calved, that had not had a heat period by 27 months of age, or had had a reoccurrence of a heat period after that age. It was believed that if such animals should become pregnant after feeding sprouted oats, it would definitely indicate that sprouted oats had value in correcting sterility in dairy cattle.

In the case of 3 of these cows, 2 of which had had 3 previous services and 1 had had 2, the service preceding the beginning of oat feeding by 2, 3, and 4 days, respectively, resulted in pregnancy, indicating a value in possibly preventing reabsorption of the fetus.

One sterile heifer that had had 3 services previous to feeding oats was fed sprouted oats for 68 days, during which time no symptoms of being in heat were observed; but after oat feeding had been stopped, there were 4 more heat periods at each of which she was bred but pregnancy did not result. An examination by a veterinarian failed to show any pathological conditions in the reproductive organs of this heifer.

Three other cows in this group seemingly were not benefited by feeding oats, but for reasons of economy it was necessary to dispose of them.

EXPERIMENTS WITH SWINE

EARLY OBSERVATION TESTS

The feeding of sprouted oats to sows was started early in 1928, about 1 year after observation tests along this line were started with cows. Sprouted oats were fed at the rate of 1 pound (dry basis) per sow per day to sows that had an abnormal breeding schedule until the sows were believed to be pregnant. Eighteen such sows were oat-fed, 5 of which later proved to be pregnant by a service which preceded the oat-feeding period. Of the other 13 sows, 11 produced litters, and with 7 of these sows there is some indication that the feeding of sprouted oats was helpful. Oats were fed for an average of 106 days. The other 4 sows that produced litters did not conceive till an average of 134 days after oat feeding had been discontinued. The two that did not produce litters were fed sprouted oats for 202 and 195 days, respectively, but to no avail.

The abnormal or irregular breeding schedule mentioned above of the 9 sows consisted in their not having any observed heat periods and, of the other 4 sows, in having repeated heat periods after having been bred once, or in some cases, several times.

LATER EXPERIMENTS

The observation tests seemed slightly favorable to feeding sprouted oats, so a more definite experiment was planned November 1, 1930, which provided for control as well as oat-fed animals and also classes for different types of breeding irregularities.

Class 1, gilts 10 months or older that seemingly did not come in heat. Such gilts were fed sprouted oats from the time they were 10 months old till 50 days after the boar service which seemed to have resulted in pregnancy.

Class 2, sows that had not been observed in heat 4 months after farrowing. Such sows were fed sprouted oats till 50 days after the last service by the boar.

Class 3, sows that had been bred once but came in heat again. Such sows were fed sprouted oats beginning at the first heat period following the one when the sow was bred, and oat feeding was continued till 50 days after the last boar service.

Class 4, sows in the control groups of classes 1, 2, and 3, that had failed to breed after a long period.

The first animal which fitted into one of the three classes was fed sprouted oats, the second was not fed oats but was considered as a control, the third sow was oat-fed, etc. Occasionally, for various reasons, some deviation from this plan was necessary. Both the oat-fed and the control animals were generally fed at least 1 pound of green alfalfa or other green feed per animal daily.

Oats were germinated in trays and fed when the sprouts were from one-half to 2 inches long at the rate of 1 pound (dry basis) per sow per day.

The results in class 3 are shown in table 4. During the period of this experiment, November 1, 1930, to the end of 1934, all of the gilts had heat periods by the time they were 10 months old, so there were no animals that fitted into class 1. Only 1 sow in class 2 was fed oats, and she had a heat period and was successfully bred 2 days after oat feeding was started. In the control group 3 sows not fed oats were successfully bred at the first observed heat period 141, 143, and 193 days, respectively, after the previous farrowing date.

TABLE 4. - Record of individual sows in class 3

Group	Sow no	Date of last previous parturition	Period from farrowing to service that resulted in pregnancy	Period oats were fed		Boar services		Litter produced
				Before pregnancy	After pregnancy	Before feeding oats	After feeding oats	
			Days	Days	Days	Number	Number	
Fed oats	84T	Aug. 31, 1929	---	121	0	3	4	No
	103T		---	20	50	3	1	Yes.
	105B	July 7, 1931	131	0	50	2	0	Yes.
	110T	Nov. 28, 1931	153	17	50	2	1	Yes.
	113B		---	16	50	2	1	Yes.
	114B		---	0	47	2	0	Yes.
	111T	June 9, 1932	147	0	50	2	0	Yes.
	114B	Nov. 27, 1932	158	32	50	2	1	Yes.
	121B		---	0	50	2	0	Yes.
	123B		---	39	51	2	2	Yes.
	124T	Sept. 5, 1933	173	73	50	2	3	Yes.
	125T		---	82	0	5	---	No.
	114B	July 29, 1933	381	71	0	2	5	Yes.
	121B	May 10, 1934	138	0	58	2	0	Yes.
	87T	Feb. 24, 1931	---	---	---	2	---	No.
	103T	Dec. 1, 1931	120	---	---	2	---	Yes.
	112B		---	---	---	2	---	Yes.
Control	115B		---	---	---	4	---	No.
	105B	Oct. 10, 1932	---	---	---	2	---	Yes.
	124T		---	---	---	6	---	Yes.
	106B	Mar. 30, 1933	195	---	---	4	---	Yes.
	118T	Mar. 14, 1933	156	---	---	3	---	Yes.
	109T	July 13, 1933	112	---	---	5	---	No.
	23T		---	---	---	6	---	Yes.
	118T	Dec. 9, 1933	254	---	---	2	---	Yes.
	123B	do	135	---	---	3	---	Yes.
	109T	Mar. 3, 1934	197	---	---	3	---	Yes.

¹ Oats not fed.

Of the 14 oat-fed sows in class 3, 12, or 86 percent, produced litters; of the 13 controls, 9, or 69 percent, produced litters. The sows in the former group were fed oats an average of 73 days each, and for the

12 sows that produced litters, an average of 3.25 services were required per conception. In the control group, the 9 sows that produced litters required 3.44 services per conception.

During the period of the experiment, 1 sow appears twice and 1 sow three times in the oat-fed group. Two sows each appear twice in the control group, and four different sows are found in both groups. This is possible since a period of about 4 years elapsed during the time of the experiment.

Of the oat-fed group, 6 sows were fed sprouted oats both before and after pregnancy; 3 were oat-fed only before, and 5 only after pregnancy.

For the sows that had previous litters, the average number of days that elapsed between the previous farrowing date and the service that finally resulted in pregnancy was 183 and 167 days, respectively, for the oat-fed and the control groups.

While the farrowing percentage is a little higher with the oat-fed sows, there is little else in these data to indicate that the feeding of sprouted oats was particularly helpful in correcting sterility or reducing the time required for conception to occur.

Class 4, which was started in January 1932, included all sows in the control groups that had not had an observed heat period or had a reoccurrence of a heat period 7 months or later after farrowing. These sows were fed sprouted oats till 50 days after the occurrence of the last heat period.

Of 5 sows that had failed to become pregnant while in the control groups and hence were put in class 4, 3 produced litters, the average period of feeding oats being 51 days. Two others that were fed oats for 74 and 132 days, respectively, failed to conceive and were finally sold.

SUMMARY AND CONCLUSIONS

It has been suggested that sprouted oats may contain a vitamin or other substance which perhaps because it prevents reabsorption of the embryo or other beneficial action, causes animals that are temporarily sterile to again become pregnant.

Early experiments with feeding sprouted oats to such animals gave rather favorable results. For lack of controls, however, definite conclusions were not possible and an experiment was planned in which every other breeding animal exhibiting a certain abnormal breeding behavior was fed sprouted oats and the alternating animals were placed in control groups.

Of 38 cows with an irregular breeding behavior, to which sprouted oats were fed, 31, or 82 percent, produced calves; of 37 cows with the same irregular breeding behavior that were put in the control group, 28, or 76 percent, produced calves.

Although in the case of some cows the feeding of sprouted oats seemed definitely helpful, equally good results were secured where oats were not fed. In general, this experiment does not demonstrate any definite value of sprouted oats for correcting sterility in cows.

Of 15 oat-fed sows, 13, or 87 percent, produced litters; of 16 control animals, 12, or 75 percent, produced litters. In addition, 3 of 5 sows that were fed sprouted oats after having failed to conceive for a long period in the control groups finally produced litters. While there is some indication here that feeding sprouted oats was of some

value when fed to shy breeders, the evidence is by no means conclusive.

All cows in the university herd receive ample green feed daily, and all sows in the university piggery, including both oat-fed and control groups were generally fed at least 1 pound of green alfalfa or other green feed per sow per day. If sprouted oats contain some substance resulting in beneficial action to shy breeders, it is possible that the same substance may be found in other green feeds. The feeding of sprouted oats to shy breeders might show more definite results if other green feeds were withheld from the experimental animals.

STUDY OF THE REMOVAL OF SPRAY RESIDUES FROM APPLES¹

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INTRODUCTION

The problem of the removal of spray residues from apples is of comparatively recent origin in Pennsylvania, where climatic conditions and a relatively light infestation of the codling moth until 1929 combined to permit the production of clean fruit with few spray applications. Since that time, however, the codling moth populations in the commercial apple-growing centers have increased to such an extent that heavier and more frequent applications of arsenicals are now necessary. A schedule calling for five cover sprays in 1932 caused some crops to show residues of arsenic in excess of the tolerance. In 1933 restrictions were placed on lead, regarding the removal of which little was known. During the 1933 season, therefore, spraying was reduced to a minimum, with the result that excessive residues were avoided at the cost of an alarming increase in codling moth damage. It became clearly evident that intensive spraying would be necessary in 1934, and that the apple crop would doubtless require washing to meet residue tolerances.

While the fundamental facts concerning the removal of spray residues from apples are rather well understood, the fact that conditions peculiar to a locality do exist makes generalization impossible. In addition, the recent developments in methods for the accurate determination of small amounts of lead make possible a more thorough understanding of the behavior of this element under washing conditions.

During the season of 1934 a program of research in methods of apple washing was instituted, the results of which are reported in this paper.

The work was planned to give information on the method best suited for the removal of spray residues from apples grown under Pennsylvania conditions. A number of factors of climate and locality are obviously variable in any study of this kind. The extended investigations on spray-residue removal conducted at other institutions, therefore, are valuable only insofar as they can be applied to local conditions; hence some of these studies repeat, for Pennsylvania, work which has been done elsewhere. It will be noted later that certain conclusions and recommendations based on investigations in other localities do not apply here.

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² The authors express their appreciation to Merrill Wood for technical assistance and to F. N. Fagan and A. W. Clyde for the use of the facilities of the Departments of Horticulture and Agricultural Engineering in this work. The authors also are indebted to S. W. Frost for the use of laboratory space, and to the fruit growers who contributed apples for experimental purposes.

EXPERIMENTAL CONDITIONS AND PROCEDURE

Part of the work was done in a laboratory set up in the largest commercial apple-growing section of Pennsylvania, in the south-central part of the State. The samples, for the most part, were collected with the cooperation of the growers of that section, but some were collected from the college orchard from experimentally sprayed trees; the work on these samples was conducted in the laboratories at State College, Pa. Over 500 samples were experimentally washed.

The varieties of apples were as follows: York Imperial, Stayman Winesap, Grimes Golden, Jonathan, Rome Beauty, Smokehouse, Delicious, Ben Davis, Stark, Hubbardston, and Yellow Newtown. The latter five varieties were represented by only a few samples.

The mean temperature for the south-central section of the State was slightly above normal during the season of 1934 except during August. The rainfall records taken at Arendtsville indicated a total precipitation for the summer months as follows: June, 6.04 inches; July, 2.51 inches; August, 5.96 inches; September, 14.09 inches. On each of 6 days during September, 1 inch or more of rain fell; the rainfall totaled 7.29 inches on 4 consecutive days, September 14 to 17, reaching nearly cloudburst proportions with 3.48 inches on September 17. The figures indicate nearly normal rainfall during June and July, when most of the codling-moth spraying was done, and a large excess in August and September, most of this coming in heavy showers during the latter month. Thus in 1934 spray applications were subjected to an average amount of weathering during the spraying period and to a great excess of rainfall between the time of the last application and the date of harvest, especially for the late varieties. It is questionable that heavy concentrated downpours of rain are as effective in removing spray residues as are more gentle rains scattered throughout the growth period of the fruit. Samples analyzed both before and after the heavy September rains showed surprisingly little reduction in spray residues from this cause. It would appear probable, therefore, that the distribution of rainfall rather than the total quantity has the greater effect on residue levels at the time of harvest.

Samples were taken by the authors, usually from the lower limbs of trees in the orchards. A sufficient number of trees were sampled to give a representative lot of apples of as nearly the minimum packed size as could be determined at the time of picking. An attempt was made to select apples of a fairly uniform size. It is well known that the smaller apples from the lower limbs of the trees usually carry the greatest amount of spray residue. When necessary, apples were taken from picking crates, but the same care was exercised in selecting for size and uniformity. Most samples were washed within 10 days after picking.

In the analysis of apple samples for lead, the method of Frear and Haley (3)³ was used, while arsenic was determined by the Gutzeit method, as described in the official methods (1, pp. 306-309). Both of these methods have been found satisfactory, although the accuracy of the lead determination is apparently greater than that of the arsenic estimation. The figures for the latter element are the mean of at least two determinations. The relative accuracy of the lead and

³ Reference is made by number (italic) to Literature Cited, p. 73.

arsenic methods, as well as the accuracy of sampling of apples, will be discussed elsewhere.

The apple washers used were of two types: One was a flotation type, similar to the washer designed by Jennings,⁴ but with structural modifications. These modifications did not affect the operation of the machine, the principle of operation of which was similar to several other types of flotation washers (2, 4). The other washer was a commercial underbrush type of the latest model, in which the washing solution is violently agitated and thrown over the apples as they advance over roller brushes. This machine was fitted with a roller drier, while the flotation machine was not equipped with a drying apparatus.

EFFECT OF VARIOUS FACTORS ON EFFICIENCY OF RESIDUE REMOVAL

The experimental work was divided into a study of the effects of the following factors on the efficiency of apple washing: Type of washer, type of washing solution, wetting agents, the use of heated solutions, spraying materials used, time of application, and variety of fruit.

EFFECT OF TYPE OF WASHER

In this study only two types of washer were available for most of the comparisons, the flotation and the underbrush type.

Table 1 gives the data secured by washing two varieties of apples sprayed with three different spray mixtures in flotation and brush machines. While individual samples showed considerable variation, a statistical analysis of the figures presented showed the underbrush washer to be significantly more efficient than the flotation washer in removing lead; the underbrush machine also was more efficient in the removal of arsenic, but the difference was not so pronounced.

The six samples identified in this and subsequent tables as 1, 2, and 3, are of both the York Imperial and Stayman Winesap varieties. They were secured in the college orchard and were treated as follows: Sample 1 received 6 cover sprays of 3 pounds of lead arsenate plus 8 quarts of lime-sulphur solution in each 100 gallons; sample 2 received 6 cover sprays of the same material, to which was added 2 pounds of skim-milk powder per 100 gallons; sample 3 received 6 cover sprays each containing 3 pounds of lead arsenate, 5 pounds of flotation sulphur and 1 quart of fish oil (cold-pressed menhaden, with less than 2 percent free fatty acids) in each 100 gallons of spray.

The underbrush washer was also more effective than the flotation washer when the hydrochloric acid solution was used with a wetting agent, as shown in table 2. In these studies the period of exposure to the acid was 1 minute in the flotation washer, and 40 seconds in the underbrush machine.

EFFECT OF DIFFERENT WASHING SOLUTIONS

Several washing solutions were tested for their efficiency in removing arsenic and lead residues from apples.

⁴ JENNINGS, B. A. THE CORNELL APPLE WASHER. Cornell Mimeograph Bull. 279, 14 pp. 1934.

TABLE 1.—Comparative efficiency of lead and arsenic spray residue removal from York Imperial and Stayman Winesap apples by means of flotation and under-brush washers, various percentages of hydrochloric acid being used

YORK IMPERIAL, LEAD

Percentage of hydrochloric acid in wash	Temperature (° F.)	Grain of lead or arsenic per pound of fruit in—					
		Sample 1		Sample 2		Sample 3	
		Flotation-washed	Brush-washed	Flotation-washed	Brush-washed	Flotation-washed	Brush-washed
Before washing		0.044	0.044	0.054	0.054	0.060	0.060
1	60	.016	.014	.016	.018	.025	.020
2	60	.020	.016	.012	.009	.020	.021
1	100	.019	.009	.015	.010	.016	.013
2	100	.013	.016	.012	.009	.021	.021
Average	---	.017	.014	.014	.012	.021	.019

STAYMAN WINESAP, LEAD

Before washing		0.049	0.049	0.075	0.075	0.042	0.042
1	60	.016	.015	.018	.012	.022	.018
2	60	.016	.011	.010	.018	.020	.018
1	100	.021	.008	.020	.014	.013	.012
2	100	.012	.013	.014	.016	.016	.014
Average	---	.016	.012	.016	.015	.018	.016

YORK IMPERIAL, ARSENIC TRIOXIDE

Before washing		0.015	0.015	0.022	0.022	0.020	0.020
1	60	.004	.003	.006	.006	.010	.006
2	60	.006	.003	.004	.003	.006	.005
1	100	.004	.003	.006	.003	.004	.005
2	100	.003	.003	.003	.002	.005	.004
Average	---	.004	.003	.005	.004	.006	.005

STAYMAN WINESAP, ARSENIC TRIOXIDE

Before washing		0.015	0.015	0.022	0.022	0.017	0.017
1	60	.003	.005	.004	.004	.007	.007
2	60	.004	.002	.003	.003	.005	.005
1	100	.003	.004	.004	.006	.005	.005
2	100	.002	.001	.003	.004	.005	.004
Average	---	.003	.003	.004	.004	.006	.005

SODIUM SILICATE

Since sodium silicate has been used with considerable success in the apple-growing sections of the Pacific coast, an attempt was made to determine its practicability under local conditions. It was found that a cold solution of sodium silicate did not remove any appreciable amount of residue when used at a concentration of 80 pounds per 100 gallons, and even when heated to 100° F. was not so effective as hydrochloric acid. It may be mentioned at this point that few Pennsylvania growers have found it necessary to use oil sprays to any extent, and the beneficial effect of an alkaline wash is observed most readily when heavy oil applications have been made to the fruit.

TABLE 2.—Effect of wetting agents on the efficiency of lead and arsenic spray-residue removal from York Imperial and Stayman Winesap apples by means of flotation and brush washers, a 2-percent hydrochloric acid wash solution being used

FLOTATION WASHER, 1 MINUTE

Sample	Grains per pound of fruit of—					
	Lead			Arsenic trioxide		
	Original load	After 2 percent HCl washing	After 2 percent HCl washing + wetting agent B ¹	Original load	After 2 percent HCl washing	After 2 percent HCl washing + wetting agent B ¹
York Imperial 1.....	0.044	0.020	-----	0.015	0.006	0.002
Stayman Winesap 1.....	.049	.016	.009	.015	.004	.001
York Imperial 2.....	.054	.012	.011	.022	.004	.001
Stayman Winesap 2.....	.075	.010	.016	.022	.003	.003
York Imperial 3.....	.060	.020	.015	.020	.006	.004
Stayman Winesap 3.....	.082	.020	.016	.017	.005	.004

UNDERBRUSH WASHER, 40 SECONDS

York Imperial 1.....	0.044	0.009	0.007	0.015	0.003	0.001
Stayman Winesap 1.....	.049	.011	.013	.015	.002	.002
York Imperial 2.....	.054	.010	.009	.022	.003	.003
Stayman Winesap 2.....	.075	.018	.018	.022	.003	.003
York Imperial 3.....	.060	.016	.008	.020	.005	.002
Stayman Winesap 3.....	.082	.018	.012	.017	.005	.003

¹ A commercial defoaming agent was added to the solutions containing the wetting agents in both types of washers. As given in this and subsequent tables, wetting agent A was Areskap, 1 gallon per 100 gallons, and wetting agent B was Vatsol, 8 pounds per 100 gallons, plus 2 quarts of Degras (Antifoam no. 16).

SODIUM CARBONATE AND SOAP

A commercial preparation composed of sodium carbonate and a coconut-oil soap was tried in various concentrations, but was not considered effective enough to warrant extended investigation.

HYDROCHLORIC ACID

Since dilute solutions of hydrochloric acid are the most widely used washing liquids, a large number of washings were made with this material. It was found that among the solutions tested it was the most effective in reducing the amount of the residues. The concentration necessary to use on various varieties showing different levels of residue was studied in some detail.

A large number of lots of apples of different varieties under various spray treatments were run through the flotation washer at room temperature, and the quantity of lead and arsenic removed by using hydrochloric acid at three concentrations was determined. The time of immersion in the washer was 1 minute; and the concentrations are given in percentage by weight of hydrochloric acid. Table 3 shows the relative effectiveness of the three concentrations in removing lead and arsenic. The results are expressed in percentage of residue (lead or arsenic trioxide) remaining on the fruit.

Maximum removal was effected by the highest concentration, and only at this concentration was the removal of arsenic proportionately as great as that of lead. This important consideration has apparently

not been mentioned in previous studies. Based on an average of all samples from various sources, the greatest efficiency of removal of both lead and arsenic was apparently at an acid concentration of approximately 2 percent by weight, when the period of immersion was 1 minute, in a flotation washer. These generalized relationships may be changed by various factors, as will be shown later.

TABLE 3.—Percentage of original arsenic and lead spray residue remaining on apples¹ after 1-minute exposure in flotation washer, various percentages of hydrochloric acid being used

Hydrochloric acid strength	Number of samples	Original residue remaining on fruit after washing	
		Lead	Arsenic trioxide
		Percent	Percent
0.5.....	10	44.5	61.5
1.0.....	74	33.1	37.9
2.0.....	17	29.5	26.8

¹ See p. 62 for varieties represented in this table.

HYDROCHLORIC ACID AND SALT

The addition of salt to solutions of hydrochloric acid has been advocated by several workers (2, 7). Overley et al. (6), however, indicate questionable results at temperatures less than 110° F. An investigation of the possible usefulness of salt was undertaken. The figures in table 4 indicate that when small quantities of salt (1 percent) are added to dilute acid in a flotation washer the efficiency of the acid is not appreciably increased. When larger quantities of salt are added there is a decided decrease in the amount of lead removed, while the amount of arsenic removed is very slightly increased.

TABLE 4.—Effect of added sodium chloride on the efficiency of lead and arsenic spray-residue removal from apples with hydrochloric acid wash solution

Composition of wash solution	Number of samples	Average lead residue remaining on fruit after washing with—		Average arsenic trioxide residue remaining on fruit after washing with—	
		Acid alone	Acid +NaCl	Acid alone	Acid +NaCl
		Percent	Percent	Percent	Percent
1 percent HCl containing 8 pounds NaCl per 100 gallons.....	2	26	26	50	45
1 percent HCl containing 50 pounds NaCl per 100 gallons.....	3	28	35	25	21
1 percent HCl containing 100 pounds NaCl per 100 gallons.....	3	28	35	25	21
2 percent HCl containing 100 pounds NaCl per 100 gallons.....	3	23	27	28	26

MIXED ACIDS

A mixture of 1 percent of hydrochloric acid and 0.5 percent of nitric acid was tried as a residue-removing solution. No apparent benefit was secured by the addition of nitric acid.

EFFECT OF WETTING AGENTS

The work of McLean and Weber in 1931 (5) indicated that the use of a wetting or foaming agent increased the effectiveness of washing in the removal of spray residues. Several commercial products have appeared on the market, and a test of their efficiency under Pennsylvania conditions was considered desirable. In using these various products, the recommendations of the manufacturer regarding the quantity to be used were followed, when such recommendations were given; otherwise a 1-percent solution by weight of the wetting agent was used. Table 5 gives typical results obtained. The data given in this table indicate that at least for the conditions of this test, these two wetting agents (the commercial preparations available to the apple industry) showed no consistent benefit.

TABLE 5.—Effect of 2 commercial wetting agents on the efficiency of lead and arsenic spray-residue removal from several varieties of apples, 1- and 2-percent hydrochloric acid wash solutions being used

Variety of apples	Grains per pound of fruit of—					
	Lead			Arsenic trioxide		
	Original load	After HCl washing	After HCl washing + wetting agent	Original load	After HCl washing	After HCl washing + wetting agent
Jonathan.....	0.140	0.033	0.034	0.024	0.013	0.012
Grimes Golden.....	.080	.026	.018	.023	.013	.011
Smokehouse.....	.070	.020	.018	.017	.008	.009
Hubbardston.....	.048	.028	.024	.018	.012	.009

1 PERCENT HCl AND WETTING AGENT B						
Jonathan.....	0.140	0.033	0.029	0.024	0.013	0.017
Do.....	.098	.033	.036	.025	.013	.013
Grimes Golden.....	.083	.026	.030	.030	.011	.015
Smokehouse.....	.054	.014	.015	.028	.011	.006
Hubbardston.....	.084	.042	.043	.038	.021	.026

2 PERCENT HCl AND WETTING AGENT A						
Jonathan.....	0.140	0.026	0.032	0.024	0.006	0.018
Do.....	.088	.023	.022	.043	.008	.009
Grimes Golden.....	.084	.020	.025	.038	.007	.011
Do.....	.073	.020	.017	.025	.007	.011
York Imperial.....	.122	.030	.022	.048	.009	.013
Stayman Winesap.....	.038	.008	.008	.013	.003	.004

2 PERCENT HCl AND WETTING AGENT B						
Jonathan.....	0.088	0.023	0.024	0.043	0.006	0.011
Grimes Golden.....	.084	.024	.024	.032	.011	.010
Do.....	.073	.020	.024	.025	.007	.018
York Imperial.....	.124	.037	.031	.038	.008	.020
Stayman Winesap.....	.038	.008	.009	.013	.003	.006

¹ Wetting agent used with 1 percent NaCl according to manufacturer's recommendations.

² Double manufacturer's recommendations.

A further study of the effectiveness of wetting agents was conducted on apple samples dipped in a smaller amount of washing solution, closely simulating in every way the treatment secured in a flotation washer. These results are shown in table 6. The apples used in this experiment received six cover sprays each containing 3 pounds of lead arsenate, 5 pounds of flotation sulphur, and 1 quart of fish oil in each 100 gallons of spray. There was no significant increase in removal of

the residue when the wetting agents were used in addition to the acid at room temperature, and a slight but consistent apparent increase in the percentage removal when wetting agent A was used with acid solution at 100° F.

TABLE 6.—*Effect of commercial wetting agents on the efficiency of lead and arsenic spray-residue removal from York Imperial and Stayman Winesap apples, when hand-dipped in 1 percent hydrochloric acid wash solution at different temperatures*

Washing treatment ¹	Grain of residue per pound of fruit for—			
	York Imperial		Stayman winesap	
	Lead	Arsenic trioxide	Lead	Arsenic trioxide
Original load.....	0.095	0.038	0.070	0.025
1 percent HCl, 1 minute at 60° F.....	.024	.010	.014	.005
Plus wetting agent A.....	.018	.007	.015	.006
Plus wetting agent B.....	.024	.012	.012	.005
1 percent HCl, 2 minutes at 60° F.....	.015	.009	.012	.004
Plus wetting agent A.....	.013	.004	.009	.003
Plus wetting agent B.....	.011	.005	.011	.004
Plus wetting agent C.....	.020	.011	.012	.006
Plus wetting agent D.....	.016	.007	.024	.010
Plus wetting agent E.....	.015	.008	.020	.007
Plus wetting agent F.....		.004	.014	.004
1 percent HCl, 1 minute, at 100° F.....	.010	.005	.008	.003
Plus wetting agent A.....	.008	.001	.005	.001
Plus wetting agent B.....	.008	.003	.010	.007
Plus wetting agent C.....	.016	.006	.012	.006

¹ Wetting agents C, D, E, and F were experimental products of the Rubber Service Laboratories Co.

As a means of determining the possible effect of the type of washer on the efficiency of the wetting agent, samples of apples were washed both in the flotation washer and in the underbrush washer, a 2-percent hydrochloric acid solution and wetting agent B being used. These results have already been presented in table 2. It is apparent that there was no very decided advantage in the use of wetting agents in either washer, although the apples sprayed with fish oil (treatment 3) showed a slightly greater removal of lead when the wetting agent was used than when the acid alone was used. From all three spray residues the removal of arsenic was apparently slightly aided in some cases by the presence of the wetting agent.

On the whole, under the conditions of these tests, the use of wetting agents did not assure an increase in the amount of spray residues removed. However, some of the data showed that additional arsenic was removed when wetting agents were employed, thus indicating that in certain cases their use may be justified. The exact conditions under which wetting agents may be expected to yield benefits, however, were not revealed in this study.

EFFECT OF RAISING THE TEMPERATURE OF WASHING SOLUTION

The use of heated solutions in the washing of apples has been recommended by several investigators. Particularly when the residue present on the fruit is extremely large, the use of a warm washing solution theoretically should be one of the most economically effective methods of removal. Studies were made comparing the efficiency of hydrochloric acid wash at approximately 60° and 100° F. The complete data have been given in table 1. The percentages remain-

ing, given in table 7, indicate the average amount of residue remaining on the three samples of each variety studied.

TABLE 7.—Comparative efficiency of lead and arsenic spray-residue removal from York Imperial and Stayman Winesap apples by 1- and 2-percent hydrochloric acid wash solutions, used in flotation and underbrush washers at 60° and 100° F..

Type of washer and variety of apple	Average residue remaining on fruit after washing with—							
	1 percent HCl at 60° F.		1 percent HCl at 100° F.		2 percent HCl at 60° F.		2 percent HCl at 100° F.	
	Lead	Arsenic trioxide	Lead	Arsenic trioxide	Lead	Arsenic trioxide	Lead	Arsenic trioxide
Flotation:	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
York Imperial.....	36	35	33	25	33	23	29	20
Stayman Winesap.....	28	26	22	23	23	20	21	19
Underbrush:								
York Imperial.....	33	26	20	18	29	20	29	16
Stayman Winesap.....	23	31	17	28	23	19	22	16

These figures show that under the conditions of this experiment the acid solutions when heated to 100° F. were definitely more effective in removing both arsenic and lead than were the corresponding acid solutions at 60°. The effectiveness of the 1-percent hydrochloric acid solution at 100° was equal to that of the 2-percent hydrochloric acid solution at 60°. The difference in ease of removal from York Imperial and Stayman Winesap, which will be discussed later, is brought out in this table.

The data on the washings in the brush machine shown in table 7 are not as consistent as might be desired. This is in part explained by the fact that with the facilities available it was not possible to maintain a constant temperature in the underbrush washer because of the violent agitation and the consequent rapid loss of heat. In the flotation washer the temperature was constant.

EFFECT OF SPRAY MATERIALS APPLIED

The ease of removal of any given spray deposit from the surface of an apple is undoubtedly affected greatly by the factors which determine the nature of this deposit. These may be complicated by a great number of variable conditions, viz., temperature, humidity, wind velocity—inasmuch as it affects speed of drying—rainfall, the chemical composition of the spray mixture applied, and the nature of the apple surface.

It was impossible, in this study, to determine the effects of all these factors, but some attention was given to the effect that the gross composition of the spray mixture may have upon the ease of removal. The data in tables 1 and 2 show the relative ease of removal of three basic spray mixtures on two varieties of apples.

The figures in table 1 have been summarized in table 8 for the three spray treatments. The percentages in the table are true averages of the four washing treatments given to fruit of each variety receiving the three different spray mixtures.

TABLE 8.—*Effect of the gross composition of the original spray used on the efficiency of arsenic and lead spray-residue removal from York Imperial and Stayman Winesap apples with hydrochloric acid wash solution used in flotation and under-brush washers*

Variety and sample no.	Original residue remaining on fruit after washing					
	Lead			Arsenic trioxide		
	Flotation machine	Under-brush machine	Average	Flotation machine	Under-brush machine	Average
	Percent	Percent	Percent	Percent	Percent	Percent
York Imperial, 1.....	39	31	35	29	20	24
York Imperial, 2.....	26	21	24	22	16	19
York Imperial, 3.....	37	31	33	31	25	28
Stayman Winesap, 1.....	33	24	29	20	20	20
Stayman Winesap, 2.....	21	20	20	16	19	18
Stayman Winesap, 3.....	22	19	20	32	31	31

These data indicate that lead and arsenic behave differently under washing treatment. Both York Imperial and Stayman Winesap fruit sprayed with lead arsenate and lime-sulphur without sticker retained the least lead residue at harvest, while the inclusion of fish oil—with flotation sulphur rather than lime-sulphur—built up the greatest deposits (table 1).

Table 8 shows that the lead residue from treatment 2 which contained skim-milk powder, offered the least difficulty in cleaning. In treatment 1 (no sticker) and treatment 3 (fish oil) more of the lead was retained after washing. In treatment 3 lead was more completely removed than in treatment 1 on Stayman Winesap, but not on York Imperial.

TABLE 9.—*Effect of gross composition and method of application of the original spray used on the ease of lead and arsenic spray-residue removal from Grimes Golden apples with 1-percent hydrochloric acid wash solution used in flotation washers*

Spray treatment (6 sprays applied) ¹	Number of samples washed	Average residue remaining on fruit after washing	
		Lead	Arsenic trioxide
		Percent	Percent
Lead arsenate, 3 pounds, lime-sulphur, 2 gallons ¹	2	30	23
Lead arsenate, 3 pounds; ² hydrated lime, 0.5 pounds, lime-sulphur, 2 gallons.....	2	39	41
Lead arsenate, 3 pounds; flotation sulphur, 5 pounds; fish oil, 1 quart.....	3	37	0
Lead arsenate, 3 pounds; skim-milk powder, 2 pounds; lethane 410, 8.5 fluid ounces; ³ lime-sulphur, 2 gallons.....	4	37	41
Lead arsenate, 3 pounds; skim-milk powder, 2 pounds; Black Leaf 40, 1 pint; ² lime-sulphur, 2 gallons; hydrated lime, 0.5 pounds ⁴	6	22	38
Lead arsenate, 3 pounds; lime-sulphur, 2 gallons; pine-tar soap, 1 pint.....	1	38	33

¹ All quantities given are per 100 gallons of spray.

² Containing casein.

³ In 2 sprays only, at oviposition peaks.

⁴ In last cover spray only.

With respect to residues of arsenic, table 1 shows that treatment 1 built up the least, and treatment 2, containing skim-milk powder, the greatest deposits at harvest. As in the case of lead removal (table

8), treatment 2 cleaned most readily. Treatment 3, however, apparently formed an arsenic deposit considerably more difficult to remove than treatment 1, this being more pronounced on Stayman Winesap than on York Imperial.

Further study was made on Grimes Golden apples which had received six different spray treatments. These samples were washed in the flotation washer with 1-percent hydrochloric acid solution. The results are shown in table 9. Among the spray mixtures containing added stickers or spreaders there were apparently no significant differences in the ease of removal; all showed higher percentages of lead and arsenic not removed by the washing treatment than was the case with the mixture of lime-sulphur and lead arsenate.

It should be mentioned again that the amount of the original load on the fruit undoubtedly affects the efficiency of removal; higher loads being removed to a proportionately greater extent than lower loads. In the case of the data in table 9, this circumstance makes the statement in the last paragraph more significant, since in all cases the load on the fruit sprayed with lead arsenate and lime-sulphur mixture was lower than when modifiers were added.

Tables 8 and 9 suggest that the different added materials had a preferential effect in the ease of removal of arsenic and lead from the three apple varieties represented. For instance, more arsenic than lead was removed from fruit of all three varieties sprayed with the mixture containing no added sticker. This was also true for the mixture containing fish oil on Grimes Golden and York Imperial, but not on Stayman Winesap. Lead and arsenic were removed with almost equal facility from the mixture containing skim-milk powder on York Imperial and Stayman Winesap, whereas on Grimes Golden the arsenic was retained to a slightly greater extent.

EFFECT OF SPRAYING SCHEDULE

The number of applications and date of the last application in relation to date of harvest have a direct bearing on residue levels and consequently on the washing treatment necessary to bring these residues below the tolerance. It was impossible to give separate attention to this phase of the problem. However, it will be noted that the original loads reported in tables 5 and 6 were in many cases very high and were not in all cases reduced below tolerance by the washing treatments applied. The fruit sampled came from various orchards heavily infested with codling moth. The Smokehouse and Grimes Golden received 6 cover sprays ending in mid-July, while most of the other varieties were sprayed 7 to 9 times between the petal-fall application and mid-August. The York Imperial and Stayman Winesap in tables 1 and 2 received 6 applications ending in mid-July. Here arsenic removal was satisfactory with cold 1-percent hydrochloric acid, even where fish oil was used in the spray mixture. Lead removal, except where fish oil was used, appeared to be equally satisfactory in most cases, though 1 sample of York Imperial failed to react to 2-percent hydrochloric acid at 60° F. and 2 samples of Stayman Winesap to 1-percent hydrochloric acid at 100°.

EFFECT OF VARIETY OF APPLES

In the reported work on the effect of varietal differences on the efficiency of spray-residue removal, the information is neither definite nor complete. Overley, St. John, Overholser, and Groves (6) state that the Winesaps cleaned more readily than did Esopus Spitzenburg, Delicious, and Yellow Newtown. Since the Stayman Winesap apples retained more spray residue at harvest than some other varieties when subjected to the same spray treatment, it was considered likely that this variety might offer more difficulty in cleaning. This proved not to be the case, as shown by the results given in table 10.

TABLE 10.—*Effect of variety of apples on the ease of lead and arsenic spray-residue removal from the fruit with 1- and 2-percent hydrochloric acid wash solutions*

Percentage of acid and variety of apple	Number of samples	Average of original remaining on fruit after washing		Percentage of acid and variety of apple	Number of samples	Average of original residue remaining on fruit after washing	
		Lead	Arsenic trioxide			Lead	Arsenic trioxide
1-percent hydrochloric acid:		Percent	Percent	2-percent hydrochloric acid:		Percent	Percent
Grimes Golden.....	20	34	32	Grimes Golden.....	6	30	19
York Imperial.....	19	36	40	York Imperial.....	5	34	35
Stayman Winesap....	17	27	38	Jonathan.....	4	28	27
Jonathan.....	6	30	39	Delicious.....	1	23	36
Rome Beauty.....	4	32	42	Stayman Winesap...	1	21	23
Smokehouse.....	3	28	32				
Ben Davis.....	1	30	32				
Delicious.....	1	38	57				
Yellow Newtown....	1	63	70				
Stark.....	1	42	33				
Hubbardston.....	1	50	55				

The factor of differential spray treatments is not considered in table 10, although it probably has considerable bearing on the removal of the residues by washing. In general, however, the type of application in these samples has been the same, lead arsenate 3 pounds to 100 gallons plus skim-milk spreader. The number of applications varied, however, from 3 to 8. No particular significance may be attached to those figures representing a single sample, but they are included in this table to indicate possible relationships which may exist between variety and ease of removal.

Table 10 shows that among those varieties of which sufficient numbers were examined to make interpretation possible, Smokehouse and Stayman Winesap cleaned most readily; Grimes Golden, Rome Beauty, and Jonathan came next while York Imperial offered considerably more difficulty.

Among the varieties represented by a single sample, Ben Davis appeared to be easy to clean, Stark offered moderate difficulty, and Yellow Newtown and Hubbardston were the two hardest to clean. Hubbardston in particular seemed to be, from other experiments, the most difficult to clean of all varieties tested.

SUMMARY AND CONCLUSIONS

This study attempted to determine the effects of various factors on the removal of arsenic and lead from apples under Pennsylvania conditions.

The removal of arsenic and lead were proportional to the concentration of hydrochloric acid, but a higher concentration of acid removed proportionately greater amounts of arsenic than lead. The concentration removing both of these elements in proportional quantities was approximately 2 percent by weight.

Wetting agents were of little value in increasing the efficiency of hydrochloric acid solutions in a flotation washer at room temperature, but a slight increase in the removal of arsenic resulted from their use in an underbrush machine. This increased efficiency was too small to be of any great importance, except possibly where fish oil was used in the spray mixture.

The variety of the apples washed was an important factor in the ease of residue removal by acid solutions. Listed in the order of increasing difficulty in residue removal, the varieties studied may be arranged tentatively as follows: Smokehouse, Stayman Winesap, Ben Davis, Grimes Golden, Rome Beauty, Jonathan, Stark, York Imperial, Delicious, while the few data available indicate that Yellow Newtown and Hubbardston are two varieties most difficult to clean.

The underbrush washer was slightly more effective in removing both arsenic and lead residues than the flotation washer, even though the time required for cleaning was less in the former.

Raising the temperature of the acid bath from 60° to 100° F. increased the efficiency, a 1-percent hydrochloric acid solution at 100° being approximately as effective as a 2-percent hydrochloric acid solution at 60°. The addition of salt, as recommended by several workers, did not increase the efficiency of acid solutions. Mixed hydrochloric acid and nitric acid solutions, sodium carbonate, and soap and sodium silicate were not so effective in lowering the residue on the fruit as dilute hydrochloric acid solutions.

Apparently the type of spray mixture applied had an effect on the ease of removal of arsenic and lead; this effect was not the same for both elements and was complicated by varietal differences. Fruit sprayed with mixtures containing a skim-milk spreader cleaned slightly more readily than did those sprayed with other combinations. The addition of fish oil to the spray mixture caused a deposit of residue more difficult to remove, but not so difficult as has been suggested in the literature.

LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 3, 593 pp., illus. Washington, D. C.
- (2) DIEHL, H. C., LUTZ, J. M., and RYALL, A. L.
1931. REMOVING SPRAY RESIDUES FROM APPLES AND PEARS. U. S. Dept. Agr. Farmers' Bull. 1687, 32 pp., illus.
- (3) FREAR, D. E. H., and HALEY, D. E.
1934. A SIMPLIFIED METHOD FOR THE RAPID DETERMINATION OF LEAD RESIDUES ON APPLES. Pa. Agr. Expt. Sta. Bull. 304, 8 pp., illus.
- (4) McLEAN, H. C., and WEBER, A. L.
1931. MODERN METHODS OF REMOVING SPRAY RESIDUES FROM APPLES AND PEARS. N. J. Agr. Col. Ext. Bull. 87, 24 pp., illus.
- (5) ——— and WEBER, A. L.
1931. USE OF WETTING OR DEGUMMING AGENTS IN THE REMOVAL OF SPRAY RESIDUES FROM APPLES. Jour. Econ. Ent. 24: 1255-1261, illus.
- (6) OVERLEY, F. L., ST. JOHN, J. L., OVERHOLSER, E. L., and GROVES, K.
1933. LEAD AND ARSENIC SPRAY RESIDUE REMOVAL FROM APPLES. Wash. Agr. Expt. Sta. Tech. Bull. 286, 83 pp., illus.
- (7) PETTEY, F. W., SKIBBE, A., and DE VILLIERS, F.
1928. INVESTIGATIONS IN CODLING CONTROL AND REMOVAL OF SPRAY RESIDUES FROM PEARS. Union So. Africa Dept. Agr. Sci. Bull. 64, 36 pp., illus.

THE EFFECT OF NUTRITIVE STATE ON THE QUANTITY OF VITAMIN A PRESENT IN THE LEAVES OF COLEUS BLUMEI¹

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INTRODUCTION

Green leaves are known to be relatively rich in vitamin A and in its precursor, carotene. The factors involved in the synthesis of the vitamin have been studied from several angles. However, the writers have seen no data which show to what degree, if any, the nutritional state of the plant is reflected in the vitamin content of the leaf. The present investigation is concerned with this question.

The problem involves the production of distinct variations in the nutrition of the plant such as are manifested by differences in activity of growth, luxuriance of foliage, and depth of pigmentation. In order to obtain the necessary leaves for assay, two distinct methods of growing the experimental plants suggested themselves. Healthy and stunted plants can, of course, be grown very easily by planting cuttings in rich compost soil and in sand, respectively. The possibility of inducing two distinct planes of nutrition simultaneously in a single plant by subjecting its parts after division to different nutritive media was considered. With this method of control, the relative quantity of vitamin A in leaves obtained from branches of the same stock, the same age, but of different development could be determined. Such an experimental procedure, however, called for the assurance that no cross transfer of food nutrients occurs from one side of the plant to the other. Certain experiments described by Auchter (1)² point to the possibility of producing differentiated growth in a single plant. His data show that the foods manufactured on one side of a plant are used and stored in that portion of the plant or are translocated to the roots directly beneath. Magness (5) similarly postulates no cross transfer of food nutrients in woody plants like the apple, for he found that trees which were half defoliated formed very few fruit buds on the defoliated sides, whereas there was a normal development of fruit buds on the undefoliated parts.

On the basis of these reports, it seemed that the vitamin assay of leaves taken from laterals of a plant divided into two parts and so planted that each side received its nutrients from different media offered a unique means for enlarging the study of the problem of the relation of the nutritional state to the quantity of vitamin A synthesized in certain organs of the plant. The present investigation was therefore undertaken to demonstrate the relation of the nutritional state to the concentration of vitamin A in the leaves. The leaves

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² Reference is made by number (italic) to Literature Cited, p. 81.

were taken from undivided cuttings grown in soil, from those grown in sand, and from the two laterals of a plant whose stem and root system had been split so as to allow the planting of each side in soil and in sand, respectively.

EXPERIMENTAL PROCEDURE

PREPARATION OF PLANT MATERIAL

Many of our food plants have only one growing point, or the laterals arise from the axils of leaves alternately or in whorls, a system of development that makes impossible the objective of securing two branches of the same age. For the present study, therefore, a plant having an opposite leaf system with laterals developing simultaneously from the axil of opposite leaves was desired, whose growth could be effectively confined to two opposite laterals arising from the same node by the pinching back of all axillary branches that might appear. No food plant on hand met the necessary requirements. *Coleus blumei*, variety Golden Bedder, on account of its structure and habit of growth, was therefore chosen.

Three series of plants were prepared for the production of leaves for the assay. The plants composing each series were propagated by rooting stem cuttings in sand. After a sufficient root system had formed, each was potted in a small pot containing rich compost soil. After the plants were well rooted and growing, the main stem was pinched back to a strong node in order to secure branching at that point. When the root system was well developed, but before the plants became pot-bound, they were removed from the containers. All soil and organic matter were washed from the roots with a gentle stream of water. The plants composing series A were planted in clay pots 4 inches in diameter which contained rich compost soil; those in series B were grown in river-washed sand, known to be quite free from inorganic matter, while those in series C were plants which had developed two strong-growing laterals in the axils of opposite leaves at the same node. The stem of each plant in series C was split longitudinally in the middle for a distance of 1 to 1½ inches from the base of the stem toward the top. A few small roots were destroyed by this procedure. One part of the root system of the plant was then planted in the same mixture of soil that was used in series A, the other half in the sand. For this planting, two tin cans, size 3, that had been fastened together by means of a wire were employed. Small holes were punched in each can to allow drainage. The cans were nailed to a board in order to make the containers more rigid. The arrangement is shown in figure 1, A.

The plants of each series were then grown in the greenhouse until specific effects of the three treatments on the developing plants were evident. Four to six weeks were required. At the end of this time, considerable differential growth has occurred between the two opposite branches and the two root systems of the divided plants (fig. 1, B). The lateral on the same side of the plant as the root system which had been planted in sand remained small and stunted. The leaves were small and undersized but nearly normally pigmented. The lateral on the same side of the plant as the root system which had been planted in rich soil grew luxuriantly. It was 3 or 4 times as large as the opposite branch and carried many more well-developed leaves. The differ-

entiated growth showed that mineral and nitrogenous nutrients which were absorbed on the soil side of the plant were utilized and elaborated to a large extent on that side only. Apparently, the half of the plant growing in sand did not receive a sufficient quantity of nutrients from this medium to support normal growth.

THE ASSAY OF VITAMIN IN COLEUS LEAVES

Leaves of coleus taken from the three series of plants were offered to four different lots of rats as the sole source of vitamin A in a diet that had been rendered free of that factor. One lot, which will be



FIGURE 1.—A, *Coleus* planted with the root system divided so that one portion grew in sand and the other in rich soil, whereby differential growth was produced in two laterals of the same age; B, divided *coleus* plant removed from containers, showing extent of root system in sand and soil media after 3 months of growth.

referred to later as group A, was fed on leaves obtained from undivided plants grown in rich soil. The second lot (group B) was given the leaves taken from similar plants grown in sand, the third lot (group C) on leaves from the lateral of the divided plant grown in soil, and the fourth lot (group D) on leaves from the lateral grown in sand.

A modification of Sherman and Munsell's method (6) was used for the determination of the relative quantity of vitamin A in the four types of *coleus* leaves. The details of the method were carefully standardized in the nutrition laboratory in regard to the specific re-

action of the colony to the rations fed, respectively, to the stock rats and to the experimental animals.

The rats used in the study originated from the stock colony belonging to the nutrition laboratory of the foods and nutrition subsection. The colony was composed of Wistar rats inbred by brother and sister mating for 60 generations. The animals weighed approximately 47 g and were 28 days old when placed upon the experimental ration. The animals were caged individually and were fed the following vitamin-A-free diet *ad libitum*: casein (free from vitamin A), 18 percent; starch, 56 percent; Osborne and Mendel salt mixture, 4 percent; and Crisco, 22 percent. Five-tenths of a gram of yeast, one-fifth of which had been irradiated, was fed separately to each rat every day. Upon this dietary regime the animals were depleted of their bodily stores of vitamin A in approximately 21 days. Stationary weight for a period of 5 days, accompanied by incipient xerophthalmia, was used as the index of depletion. At the end of the depletion period, the average weight of the animals was 98.5 g. The rats were divided into four groups with litter mates, males, and females distributed as uniformly as possible in each group. Coleus leaves obtained from plants grown as described above were then offered to the members of each group as the only source of vitamin A in their food.

Preliminary trials indicated that 20 mg of coleus leaf grown in sand, when fed daily, furnished approximately 1 unit of vitamin A as defined by Sherman and Munsell (6). The four kinds of coleus leaves were therefore offered at this level. In sampling, each leaf was cut in segments and numbered according to the position of the segment in the leaf. On the first day, the first member of a group received segment 1; on the second day, segment 2, and so on. With this arrangement, as the experiment progressed, all rats received representative samples from all parts of the leaf, thus error due to unequal distribution of the vitamin in the leaf was avoided. The segments were carefully weighed on a torsion balance. A double portion was offered on Saturday. Special care was taken to insure the consumption of the daily dose of the coleus leaves offered. The vitamin feeding was continued for 8 weeks.

RESULTS

Data are presented in table 1 which show that rats receiving, as the sole source of vitamin A in the diet, a daily dose of coleus leaf taken from undivided plants grown entirely in sand gained less during the experimental period than did any other group. Of particular interest is the fact that the average rate of growth of this lot of rats was approximately one-half that of the group given similar-appearing coleus leaf removed from the stunted lateral nourished by the inadequate supply of nutrients present in sand. There were slight differences in the average total gains in body weight made by the three groups of rats fed coleus leaf taken, respectively, from the undivided plant grown in soil, the divided plant grown in sand, and the divided plant grown in soil. Upon analysis, these differences were not found to be statistically significant as the data recorded in table 2 indicate.

TABLE 1.—Mean weights at end of depletion period and mean gains in 8 weeks of the 4 groups of rats fed vitamin-A-free diets supplemented by 20 mg of coleus leaf taken from plants treated in 3 different ways

Group	Treatment of plant	Rats		Weight at end of depletion period	Mean-weight gain in 8 weeks
		Started	Survived		
		Number	Number	Grams	Grams
A	Grown in soil.....	20	17	94	45.82
B	Grown in sand.....	13	8	98	22.13
C	One-half grown in soil.....	21	18	99	49.22
D	One-half grown in sand.....	23	20	103	42.90

TABLE 2.—Values of *t* between mean total gains for each series, secured by analysis of variance

Groups of rats classified according to kind of coleus fed	B, all sand	C, soil half of divided plants	D, sand half of divided plants
A, all soil.....	1 3 015	0.651	0.527
B, all sand.....		1 3.357	1 2.448
C, soil half of divided plants.....			1.122

¹ Significant.

² Highly significant.

Estimates of experimental error were obtained by analyzing the variance in the manner devised by Fisher (3). Tests of significance for the values of *t* were made by means of the table of values of *r*, *R*, and *t* adapted by Wallace and Snedecor (7) from Fisher's tables. Similar statistical treatment showed that the differences in the mean body weights of the four groups of experimental animals at the end of the depletion period (table 1) were not significant. For instance, in testing for the significance of the differences noted in mean weights of the groups at this time, a value for *t* of 0.7377 was obtained when groups A and B were compared, and of 1.102 when the mean weights of the rats in groups C and D were similarly analyzed. Inasmuch as these values do not indicate significant differences, the greater increments in growth made by groups A, C, and D in comparison with that made by group B cannot be attributed to any cause except to variation in the vitamin content of the leaves fed to the four groups of animals.

DISCUSSION

These results lead to very interesting speculation. Unmistakably, the leaves of a stunted underdeveloped plant grown in a medium extremely poor in the essential nutrients contain less vitamin A than do the leaves of similar plants grown in a highly favorable nutritive medium. Evidently some factor necessary for the synthesis of vitamin A is lacking in the nutrients provided by the sand. That the lack of certain plant nutrients may definitely retard the synthesis of vitamin A has been noted by Dutcher (8, p. 123) in unpublished data. He found that spinach made chlorotic by growing in soil containing insufficient manganese manufactured less vitamin A than did spinach grown with adequate amounts of the element.

On the other hand, a plant, one-half of which is stunted, produces small leaves on the inactively growing side that, weight for weight, are relatively as rich in vitamin A as are the large leaves from the well-nourished half of the plant. The difference in the nutritive state of the two sides of the plant seems to indicate that there is no cross transfer of food materials from one side of the plant to the other in *Coleus blumei*. Apparently, then, the common plant nutrients such as nitrogen, phosphorus, potash, calcium, etc., may not be concerned in the synthesis of the vitamin or the vitamin may have been synthesized and transferred. Auchter (1) has shown that water passes from one side of a plant to the other; but water cannot be the determining factor in producing a like vitamin content of the leaves, for the divided and undivided parts were both liberally supplied with water. However, in its passage through the plant, water may have carried from the well-nourished part of the coleus some soluble inorganic or organic material concerned with the manufacture of vitamin A, but not with growth. Or perhaps the vitamin itself or its precursor, carotene, elaborated in the adequately nourished side of the plant was transported to the poorly nourished half. The recent demonstration by Von Euler and Klusmann (3) that carotene and other carotenoid pigments pass into aqueous solutions of the bile salts suggests the possibility of a transfer of this nature. The hypothesis of a translocation of vitamin A, however, is not substantiated by certain statements of Hauge and Trost (4), if the findings obtained with ears of corn as the test material apply to the present situation. These workers crossed Reid Yellow Dent and Johnson County White Dent corn and were unable to detect any measurable transfer of vitamin A to white endosperm grains selected from F_2 segregating ears. They found that vitamin A was always associated with the yellow endosperm and that white grains when grown on the same ears with the yellow grain did not contain vitamin A.

No reasonable explanation, therefore, can be offered at present for the phenomena described. The most significant point of the experiment lies in the results obtained; i. e., an undivided plant grown in sand does not have the ability to synthesize normal quantities of vitamin A in its leaves whereas a plant, divided and growing one-half in soil and one-half in sand, is able to produce normal quantities of vitamin A in the leaves of the stunted lateral.

SUMMARY AND CONCLUSIONS

Vitamin A was not synthesized in as large quantities in a poorly nourished stunted coleus plant as it was in an actively growing, well-nourished one. However, when differentiated growth was induced in two sides of a single plant, the leaves of the underdeveloped lateral contained relatively as much vitamin as did those on the luxuriantly growing branch.

These findings suggest either a specific but unidentified nutrient factor essential for the synthesis of vitamin A or a cross transfer within the plant of potential vitamin A materials in a manner that is not characteristic of the food factors essential for the growth of the plant.

LITERATURE CITED

- (1) AUCHTER, E. C.
1923. IS THERE NORMALLY A CROSS TRANSFER OF FOODS, WATER AND MINERAL NUTRIENTS IN WOODY PLANTS? Md. Agr. Expt. Sta. Bull. 257, pp. [33]-60.
- (2) EULER, H. v., and KLUSSMANN, E.
1933. ZUR BIOCHEMIE DER CAROTINOIDE UND DES VITAMINS C (ASCORBIN-SÄURE). Hoppe-Seyler's Ztschr. Physiol. Chem. 219: 215-223.
- (3) FISHER, R. A.
1930. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London.
- (4) HAUGE, S. M., and TROST, J. F.
1928. AN INHERITANCE STUDY OF THE DISTRIBUTION OF VITAMIN A IN MAIZE. Jour. Biol. Chem. 80: 107-114, illus.
- (5) MAGNESS, J. R.
1917. STUDIES IN FRUIT-BUD FORMATION. Oreg. Agr. Expt. Sta. Bull. 146: 1-18, illus.
- (6) SHERMAN, H. C., and MUNSELL, H. E.
1925. THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Amer. Chem. Soc. 47: 1639-1646, illus.
- (7) WALLACE, H. A., and SNEDECOR, G. W.
1931. CORRELATION AND MACHINE CALCULATION. Rev. by G. W. Snedecor. 71 pp., illus. Ames, Iowa. (Iowa Agr. Col. Off. Pub. v. 30, no. 4.)
- (8) WHITE HOUSE CONFERENCE ON CHILD HEALTH AND PROTECTION.
1932. GROWTH AND DEVELOPMENT OF THE CHILD. SECTION I. MEDICAL SERVICE. PART III. NUTRITION. Report of the Committee on Growth and Development. 532 pp. New York and London.

THE PHOSPHORUS REQUIREMENTS OF DAIRY HEIFERS¹

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INTRODUCTION

An earlier paper³ in this journal dealt with the calcium requirements of growing dairy heifers. Since that report was published an investigation of phosphorus requirements has been conducted along the same general lines.

EXPERIMENTAL ANIMALS

One pure-bred and seven high-grade Holstein-Friesian heifers were used. They were divided into two groups designated as the high-phosphorus and low-phosphorus groups. Table 1 shows their history. Unfortunately, two of them (both in the same group) had to be disposed of when their experimental period was about half completed.

TABLE 1. *History of the heifers in the phosphorus-requirement tests*

Group	Heifer no.	Born	Age at commencement of experiment	First calf delivered	Age at first calving
			<i>Days</i>		<i>Days</i>
High-phosphorus ration.....	162	Aug. 29, 1929	181	Nov. 28, 1931	821
	175	Jan. 10, 1930	114	June 26, 1932	898
	190	Apr. 12, 1931	159	Sept. 18, 1933	890
	192	Apr. 10, 1931	161	Aug. 29, 1933	872
Average.....			154		870
Low-phosphorus ration.....	¹ 163	Aug. 31, 1929	179	No calf.....	-----
	¹ 168	Dec. 16, 1929	128	do.....	-----
	191	Apr. 12, 1931	159	Aug. 11, 1933	852
	193	Apr. 1, 1931	170	Aug. 19, 1933	871
Average.....			² 165	-----	862

¹ Reacted to the tuberculin test, Feb. 9, 1931; slaughtered Feb. 16, 1931.

² Average age of the two that calved.

NATURE OF THE RATIONS

The basal ration was composed of:

Mixed hay (low in phosphorus).

Dried beet pulp.

Grain mixture { 8 parts corn meal.

 { 1 part corn gluten meal.

 { 1 part blood flour.

This combination resulted in a ration quite low in phosphorus. For the high-phosphorus group it was supplemented with requisite

¹ Received for publication Mar. 4, 1935; issued August 1935. Contribution no. 188 of the Massachusetts Agricultural Experiment Station.

² The advice and suggestions of Dr. J. B. Lindsey, former head of the Department of Chemistry at this station, are acknowledged and appreciated.

³ LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. THE CALCIUM REQUIREMENTS OF DAIRY HEIFERS. Jour. Agr. Research 42: 883-896, 1931.

amounts of rice bran, a product relatively high in phosphorus. The additional amounts of other nutrients furnished by the rice bran were offset for the low group by slightly larger amounts of the basal ration.

The proportions of the several ingredients fed to the two groups at varying ages are shown in table 2. The slightly larger average daily feed intake by the high-phosphorus group is due to the fact that these animals, as a group, were slightly heavier from start to finish than those in the low-phosphorus group.⁴

The hay fed was designedly as low in phosphorus as could be purchased locally. It was grown on farms in the vicinity of the experiment station, was cut late, and came from run-out fields. The beet pulp was fed to give the ration greater palatability and also to furnish calcium. The grain mixture provided adequate amounts of protein and energy and was very low in phosphorus. The composition of the feeds is shown in table 3.

TABLE 2.—Average daily feed consumption of heifers by groups and ages

Group and age	Hay	Beet pulp	Grain mixture	Rice bran	Total daily intake
	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
High-phosphorus:					
Calves.....	5.63	0.99	3.12	0.99	10.73
Yearlings.....	9.00	1.28	3.50	1.10	14.88
2-year-olds.....	11.55	1.88	3.88	1.88	19.19
Low-phosphorus:					
Calves.....	5.94	.99	3.36	-----	10.29
Yearlings.....	9.09	1.35	3.77	-----	14.21
2-year-olds.....	12.72	1.94	3.98	-----	18.64

⁴ The method of feeding previous to placing the heifers on the experimental rations at about 5 months of age was the same for all individuals.

TABLE 3.—Minimum, maximum, and average composition of feeds used in the experiments

Different feeds	Moisture		Total ash			Crude protein			Crude fiber			Ether extract			Calcium			Phosphorus			Magnesium		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average		
Number	Pct.	t.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.		
7	9.12	10.56	10.10	3.93	6.30	4.98	5.10	7.32	6.44	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.	Pd.		
Hay	8.03	11.31	9.74	2.50	5.47	3.52	9.56	12.44	10.73	16.13	21.06	19.22	.09	.52	.86	.636	.907	.724	.051	.136	.285		
6	11.83	13.27	12.53	1.47	2.94	2.37	22.57	25.34	23.85	1.85	2.23	1.98	.88	4.59	2.86	.014	.131	.061	.265	.353	.312		
Dried beet pulp	8.03	11.31	9.74	2.50	5.47	3.52	9.56	12.44	10.73	16.13	21.06	19.22	.09	.52	.86	.636	.907	.724	.051	.136	.285		
4	11.83	13.27	12.53	1.47	2.94	2.37	22.57	25.34	23.85	1.85	2.23	1.98	.88	4.59	2.86	.014	.131	.061	.265	.353	.312		
Grain mixture	8.70	9.47	9.13	10.73	12.82	11.76	13.15	15.05	14.19	9.42	10.73	10.03	12.69	17.78	14.31	.043	.131	.062	.608	.858	.757		
5	8.70	9.47	9.13	10.73	12.82	11.76	13.15	15.05	14.19	9.42	10.73	10.03	12.69	17.78	14.31	.043	.131	.062	.608	.858	.757		
Rice bran	8.70	9.47	9.13	10.73	12.82	11.76	13.15	15.05	14.19	9.42	10.73	10.03	12.69	17.78	14.31	.043	.131	.062	.608	.858	.757		

The high-phosphorus ration supplied about one and two-thirds times as much of that element as did the low-phosphorus ration. Other constituents were kept as nearly on a par as possible (table 4). Intake of digestible protein per unit of body weight was practically identical for both groups throughout, but the high-phosphorus group received an average amount of total digestible nutrients per unit of weight slightly greater than did the low-phosphorus group. This was due to a somewhat higher content of fat in the rice bran than was anticipated. Analyses on record give the average crude fat content of rice bran as about 10 percent; the samples taken during this experiment averaged 14.3 percent.

TABLE 4.—Daily intake of total and digestible nutrients by heifers, per 100 pounds live weight

Age	Group	Dry matter	Crude protein	Crude fiber	Nitrogen-free extract	Fat	Calcium	Phosphorus	Digestible protein	Total digestible nutrient
		Lb.	Lb.	Lb.	Lb.	Lb.	Lb.	Lb.	Lb.	Lb.
Calves.....	{High-phosphorus....	2.10	0.27	0.43	1.25	0.077	0.0085	0.0072	0.17	1.51
	{Low-phosphorus....	1.96	.26	.39	1.18	.048	.0082	.0040	.16	1.35
Yearlings.....	{High-phosphorus....	1.67	.20	.36	.97	.052	.0067	.0050	.12	1.14
	{Low-phosphorus....	1.80	.21	.40	1.07	.041	.0075	.0034	.13	1.22
2-year-olds.....	{High-phosphorus....	1.66	.18	.37	.97	.055	.0065	.0045	.10	.91
	{Low-phosphorus....	1.58	.17	.36	.93	.037	.0069	.0026	.10	1.05
Weighted averages for the duration of the experiment.	{High-phosphorus....	1.78	.22	.38	1.04	.058	.0071	.0055	.13	1.22
	{Low-phosphorus....	1.71	.21	.37	1.02	.040	.0072	.0033	.13	1.16

RESULTS OF METABOLISM BALANCE TRIALS

Seventy-seven metabolism balance trials were carried out, 42 with the high-phosphorus group and 35 with the low-phosphorus group. It should be borne in mind that these metabolism-balance periods were not continuous, but were conducted at frequent intervals for 10-day periods throughout the course of the investigation. The experimental and analytical procedure was identical with that described in the earlier publication.⁵ The progress of the work has been accelerated somewhat by the addition of two more metabolism stalls, thus making it possible to have four heifers on trial at one time. Figure 1 shows the general arrangement of the stalls.

⁵ LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.

Methods of analysis were as follows:

For nitrogen the Kjeldahl method described in the following publication: ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Compiled by the committee on editing methods of analysis. Ed. 3, 593 pp., illus. Washington, D.C. 1930.

For calcium and magnesium the method of McCrudden modified for use with materials of this nature: McCrudden, F. H., THE QUANTITATIVE SEPARATION OF CALCIUM AND MAGNESIUM IN PRESENCE OF PHOSPHATES AND SMALL AMOUNTS OF IRON DEVISED ESPECIALLY FOR THE ANALYSIS OF FOODS, URINE AND FECES. Jour. Biol. Chem. 7:83-100. 1910.

For phosphorus the colorimetric method described in the following publication: FISKE, C. H., and SUBBAROW, Y. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. Jour. Biol. Chem. 66: 387-389. 1925.

The frequency with which individual heifers were used as members of the high-phosphorus and low-phosphorus groups was as follows: High-phosphorus group—heifers 162 and 175, 16 times; heifer 190, 4 times; heifer 192, 6 times. Low-phosphorus group—heifer 163, 8 times; heifer 168, 4 times; heifer 191, 11 times; heifer 193, 12 times. The uneven distribution of trials between individuals was due in the first place to the loss of nos. 163 and 168 early in their yearling period, and to a subsequent effort to even up the number of trials for the two



FIGURE 1.—Arrangement of metabolism stalls used in the experiments.

groups by using nos. 191 and 193 considerably more than nos. 190 and 192.

The detailed balance records for the individual heifers are given in table 5 and the data are summarized in table 6. The values in this table were obtained by taking the average of all trials for a given age of all individuals in the group. The probable error of these averages was determined by means of Bessel's formula, viz.,

$$P.E._m = 0.6745 \sqrt{\frac{\sum d^2}{n(n-1)}}$$

Average as a yearling	20	870	Oct. 3 to 12, 1931	15,101	3,376	2,973	1,866	3,161	1,132	692	212	20,03	23,54	23,25	11,37
	21	925	Nov. 7 to 16, 1931	14,291	3,222	2,801	1,765	3,437	1,424	283	-0.65	24,06	44,20	10,45	-3.68
	22	1,080	Dec. 26, 1931, to Jan. 4, 1932	13,921	3,510	2,670	1,744	2,971	819	221	0.15	21,34	23,34	8,28	-0.86
	23			14,816	3,000	2,477	1,684	3,102	819	584	0.67				
Average as a 2-year-old	25	1,120	Feb. 20 to 29, 1932	12,796	2,724	2,427	1,837	2,437	1,08	355	-0.28	19,04	3,88	14,62	-1.33
	26	1,190	Apr. 2 to 11, 1932	12,674	2,996	2,358	1,908	2,710	618	201	0.17	21,12	20,63	12,24	8.96
	27	1,240	Apr. 25 to May 4, 1932	12,431	2,800	2,256	1,831	2,410	605	0.64	0.040	19,39	20,86	2,38	2.16
				12,700	2,873	2,346	1,859	2,522	443	233	0.01				
90	6	340	Oct. 20 to 29, 1931	25,670	5,241	4,379	2,844	7,482	2,036	1,404	-0.200	29,15	28,84	34,12	-7.04
	7	405	Nov. 28 to Dec. 7, 1931	21,494	4,422	3,677	2,385	5,531	2,403	1,032	-0.121	26,73	54,35	28,07	-5.07
	9	490	Jan. 23 to Feb. 1, 1932	20,319	3,814	3,289	2,451	4,850	1,193	1,075	-0.146	23,87	33,02	50,93	5.96
	11	568	Mar. 19 to 28, 1932	18,317	3,490	2,945	2,255	3,591	680	555	-0.159	19,60	19,76	18,84	-7.04
Average as a calf				21,450	4,192	3,573	2,484	5,364	1,581	1,189	-0.084				
	6	390	Oct. 20 to 29, 1931	23,503	5,235	3,977	2,622	6,858	1,646	1,038	-0.132	29,18	31,45	26,09	-5.04
	7	478	Nov. 28 to Dec. 7, 1931	20,060	4,503	3,404	2,240	6,172	2,094	772	-0.068	30,77	46,80	22,69	-3.01
	8	465	Dec. 26, 1931, to Jan. 4, 1932	21,069	4,725	3,413	2,250	6,573	2,029	882	0.051	31,19	42,94	25,85	2.25
92	9	545	Jan. 23 to Feb. 1, 1932	19,120	3,647	3,071	2,354	4,726	1,038	1,559	0.001	24,72	28,45	44,24	3.87
	10	585	Feb. 20 to 29, 1932	17,771	3,382	2,850	2,186	4,651	599	922	-0.066	26,17	16,84	32,36	-1.67
	11	616	Mar. 19 to 28, 1932	16,984	3,548	2,759	2,184	3,875	739	903	-0.141	22,82	20,54	32,73	-6.47
				19,751	4,173	3,246	2,306	5,476	1,351	970	-0.039				
Average as a calf															

LOW-PHOSPHORUS GROUP

Average as a yearling	6	315	Mar. 22 to 31, 1930	20,605	3,427	1,643	1,624	6,407	0,676	0,522	-0.482	31,10	19,72	31,77	-29.65
	7	365	Apr. 19 to 28, 1930	18,532	3,515	1,510	1,566	4,184	621	563	-0.262	22,58	17,66	37,44	-16.71
	8	410	May 10 to 19, 1930	16,618	3,121	1,346	1,390	3,846	037	383	-0.293	23,28	1,20	28,42	-17.80
	9	430	June 1 to 10, 1930	15,300	3,077	1,266	1,280	4,967	971	498	-0.416	32,46	31,55	39,37	-32.32
Average as a calf				17,739	3,265	1,441	1,465	4,851	576	492	-0.352				
	13	545	Oct. 5 to 14, 1930	17,719	3,940	1,862	1,733	4,588	1,019	610	0.092	25,89	28,87	32,75	5.28
	14	600	Nov. 6 to 15, 1930	16,196	3,562	1,699	1,574	4,250	594	444	-0.067	26,24	16,67	26,15	-4.23
	15	635	Dec. 9 to 18, 1930	17,102	3,377	1,901	1,649	4,649	644	512	-0.047	27,18	19,07	26,40	-3.04
Average as a yearling	16	695	Jan. 24 to Feb. 2, 1931	15,848	3,248	1,741	1,463	4,892	609	846	-0.105	5,63	18,76	47,25	-7.18
				16,716	3,532	1,823	1,577	3,595	717	903	-0.032				

TABLE 5.—Detailed balance record for each heifer in the high- and low-phosphorus metabolism experiments, 1930-33—Continued

LOW-PHOSPHORUS GROUP—Continued

Heifer no.	Age		Weight Pounds	Date of experiment	Daily intake per 100 pounds live weight				Daily retention per 100 pounds live weight				Retention of intake			
					N	Ca	P	Mg	N	Ca	P	Mg	N	Ca	P	Mg
	Months	Days			Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Percent	Percent	Percent	Percent
Average as a calf	10	4	430	Oct. 20 to 29, 1930	18.143	3.621	1.859	1.694	4.563	883	728	.060	23.82	38.72	3.63	
	11	8	495	Nov. 24 to Dec. 3, 1930	17.494	3.168	1.921	1.494	6.146	1.994	.882	.201	36.15	31.38	50.25	13.45
	12	25	520	Jan. 10 to 19, 1931	17.814	3.395	1.880	1.579	5.356	.929	.882	.071				
	13	23	565	Feb. 8 to 17, 1931	16.729	3.052	1.842	1.433	4.969	.902	.971	.109	29.82	31.52	52.72	7.60
Average as a yearling	6	8	390	Oct. 20 to 29, 1931	17.048	3.121	1.885	1.462	4.271	.784	.870	.189	20.46	19.00	39.57	-32.64
	7	16	455	Nov. 28 to Dec. 7, 1931	23.040	5.170	2.312	1.796	8.026	1.874	.706	.081	34.83	36.24	34.43	-4.51
	8	14	490	Dec. 26, 1931 to Jan. 4, 1932	19.700	4.457	1.986	1.539	8.572	2.277	.749	.096	33.36	49.68	37.68	-2.33
	9	11	540	Jan. 23 to Feb. 1, 1932	20.075	4.702	2.043	1.580	8.267	1.963	.847	.029	31.22	42.38	41.46	1.53
Average as a calf	10	8	575	Feb. 20 to 29, 1932	18.176	3.615	1.817	1.743	4.736	1.086	.853	.094	26.06	30.08	46.94	-5.38
	11	21	620	Apr. 2 to 11, 1932	17.043	3.380	1.706	1.635	3.675	.306	.812	.109	21.54	9.04	47.59	-6.67
	12	13	664	Apr. 25 to May 4, 1932	16.346	3.451	1.658	1.629	2.907	.695	.334	.064	13.95	20.13	20.15	-3.92
	13	10	682	May 22 to 31, 1932	19.063	4.129	1.920	1.654	5.314	1.364	.732	.059				
Average as a yearling	14	0	695	June 12 to 21, 1932	15.443	3.267	1.572	1.539	3.720	1.005	.441	.065	24.00	30.77	38.06	4.25
	24	2	1,011	Apr. 14 to 23, 1933	15.178	3.166	1.535	1.503	3.110	.607	.266	.132	21.81	19.16	18.63	8.77
	25	3	1,073	May 15 to 24, 1933	14.823	3.021	1.495	1.445	2.765	.723	.529	.086	18.66	23.92	35.43	2.46
	26	3	1,073	May 15 to 24, 1933	15.148	3.151	1.534	1.496	3.265	.778	.419	.078				
Average as a 2-year old	27	7	1,011	Apr. 14 to 23, 1933	12.111	2.947	1.143	1.315	2.277	.634	.372	.086	18.80	21.82	32.56	-7.28
	28	7	1,073	May 15 to 24, 1933	12.736	3.163	1.209	1.413	2.221	1.204	.280	.106	17.44	37.70	23.20	7.47
	29	7	1,011	Apr. 14 to 23, 1933	12.424	3.070	1.176	1.364	2.249	.919	.326	.005				
	30	7	1,011	Apr. 14 to 23, 1933	22.157	4.979	2.226	1.729	7.333	2.463	.942	.209	33.05	49.48	42.34	-12.11
Average as a 2-year old	31	7	1,011	Nov. 28 to Dec. 7, 1933	19.072	4.314	1.923	1.490	5.910	1.403	.787	.175	30.98	41.89	40.96	-11.74
	32	8	510	Dec. 26, 1933 to Jan. 4, 1934	19.288	4.618	1.963	1.518	5.732	2.471	.889	.205	29.72	54.69	45.31	-13.49
	33	9	540	Jan. 23 to Feb. 1, 1934	17.970	3.519	1.789	1.707	4.107	1.128	.875	.160	22.85	32.05	48.92	-9.35
	34	10	580	Feb. 20 to 29, 1934	16.896	3.351	1.691	1.621	3.639	.728	.888	.104	21.54	21.73	52.50	-6.39

TABLE 6.—Summary of balances by ages in the high- and low-phosphorus metabolism experiments, March 1930 to June 1933

Average as a calf		19.083				4.136	1.918	1.613	5.344	1.719	.876	-.171								
12		640				3.343	1.606	1.578	2.876	.981	.413	-.106	18.16	20.34	25.74					
24		Apr. 2 to 11, 1932				15.835	3.343	1.578	2.876	.981	.413	-.106	18.16	20.34	25.74					
12		Apr. 25 to May 4, 1932				15.191	3.214	1.514	2.825	.952	.363	-.006	20.30	28.61	35.91					
13		May 22 to 31, 1932				14.937	3.116	1.511	3.037	.975	.420	-.017	20.83	31.30	37.77					
14		June 12 to 21, 1932				14.510	2.957	1.463	2.536	.782	.523	-.016	17.48	26.44	35.71					
11																				
Average as a yearling						15.118	3.158	1.532	2.819	.923	.415	-.030								
24		Mar. 27 to Apr. 5, 1933				12.669	3.003	1.193	2.078	.542	.245	-.124	16.40	18.05	20.56					
24		Apr. 29 to May 8, 1933				12.705	3.169	1.205	2.648	.635	.433	-.017	20.84	32.67	38.91					
26		June 3 to 12, 1933				12.065	3.024	1.144	1.338	1.748	1.024	-.014	14.49	33.85	26.53					
Average as a 2-year-old						12.480	3.065	1.181	1.364	2.158	.967	-.052								

TABLE 6.—Summary of balances by ages in the high- and low-phosphorus metabolism experiments, March 1930 to June 1933																				
Group and number of trials		Daily intake, per 100 pounds live weight						Daily retention, per 100 pounds live weight						Retention of intake			Ratio of Ca to P			
		N			Ca			P			Mg			N	Ca	P	Mg	Intake	Retention	
		Grams	Percent	Percent	Grams	Percent	Percent	Grams	Percent	Percent	Grams	Percent	Percent							
High-phosphorus:																				
As calves, 16 trials.....		19.85	3.75	3.25	2.27	5.46	1.36	1.01	0.05	27.51	36.37	31.08	1.16:1	1.35:1						
As yearlings, 21 trials.....		15.12	3.03	2.49	1.73	3.39	±.09	±.05	.07	22.42	26.40	±1.48	4.05	1.22:1						
As 2-year-olds, 5 trials.....		13.23	3.07	2.49	1.82	3.18	±.83	±.03	.04	24.04	27.04	±1.33	2.20	1.23:1						
Average.....		16.70	3.31	2.78	1.94	4.15	1.02	±.02	.02	24.85	30.82	±3.58	1.03	1.19:1						
Low-phosphorus:																				
As calves, 17 trials.....		18.61	3.85	1.80	1.59	5.22	1.23	.73	-.15	28.05	31.95	40.56	2.13:1	1.68:1						
As yearlings, 13 trials.....		15.91	3.27	1.68	1.52	3.38	±.12	±.03	-.03	21.24	24.46	±1.42	0	1.95:1						
As 2-year-olds, 5 trials.....		12.46	3.07	1.18	1.36	2.21	±.03	±.04	-.03	17.58	28.99	±1.89	0	2.60:1						
Average.....		16.73	3.52	1.67	1.53	4.11	1.02	.60	-.09	24.57	28.98	±2.90	0	2.11:1						

Group and number of trials

High-phosphorus:

As calves, 16 trials.

As yearlings, 21 trials.

As 2-year-olds, 5 trials.

Average.

Low-phosphorus:

As calves, 17 trials.

As yearlings, 13 trials.

As 2-year-olds, 5 trials.

Average.

RETENTION AS RELATED TO INTAKE

Considering first the results as a whole: The nitrogen, calcium, and phosphorus balances were positive in all cases. With the average intake of nitrogen per unit of weight practically identical for both groups, the high-phosphorus group retained 1 percent more, indicating slightly, but not significantly, better utilization of nitrogen by that group.

Retention of calcium per unit of weight was the same for both groups, but since the unit intake for the high-phosphorus group was 6 percent less than for the low-phosphorus group, utilization by the former was slightly, but not significantly, superior (30.8 percent as contrasted with 29 percent).

Retention of phosphorus per unit of weight was somewhat greater in the high-phosphorus group (0.73 g as compared with 0.60 g daily), but since the intake by the low-phosphorus group was only 60 percent that of the high-phosphorus group, the utilization by the low-phosphorus group was significantly greater (35.9 percent as contrasted with 26.3 percent). This is in agreement with the earlier work on calcium requirements,⁶ where it was noted that the lower intake was more efficiently utilized.

Forty-one of the magnesium balances were negative; 15 in the high-, 26 in the low-phosphorus group. Although they occurred more frequently in the low-phosphorus group, the average negative balance was not much larger for that group (-0.146 g daily per 100 pounds live weight as compared with -0.137 g daily for the high-phosphorus group). The net result for the groups as a whole was a very slight positive balance of magnesium for the high-phosphorus group and a slight negative balance of that element for the low-phosphorus group. The significance of such a large number of negative balances of an element is not readily apparent. A similar situation was noted and commented upon in the earlier paper on the subject.⁷ The departure from normal environment in the conduct of metabolism experiments, while it may not interfere to any extent with storage of elements which are utilized in relatively large proportion to their intake (e. g., nitrogen, calcium, or phosphorus), may be sufficient at times to shift to the negative side the equilibrium of an element like magnesium, which at best is utilized in relatively small proportion.

EFFECT OF AGE ON INTAKE, RETENTION, AND UTILIZATION

The average results⁸ for different ages are portrayed graphically in figures 2 and 3 in addition to being summarized in table 6. The graphs show the relative decrease (or increase) in intake, retention, and percentage utilization per unit of weight with advancing age. They were constructed by assuming in each case a value of 100 for the first year, values for the second and third years being expressed in the appropriate percentage ratio. They show quite clearly—

(1) That intake per unit of weight in general decreased with advancing age, which is normal. The rate of decrease was usually more

⁶ LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.

⁷ LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.

⁸ Because of the many negative balances, results for magnesium do not lend themselves to graphic portrayal, and hence are not included.

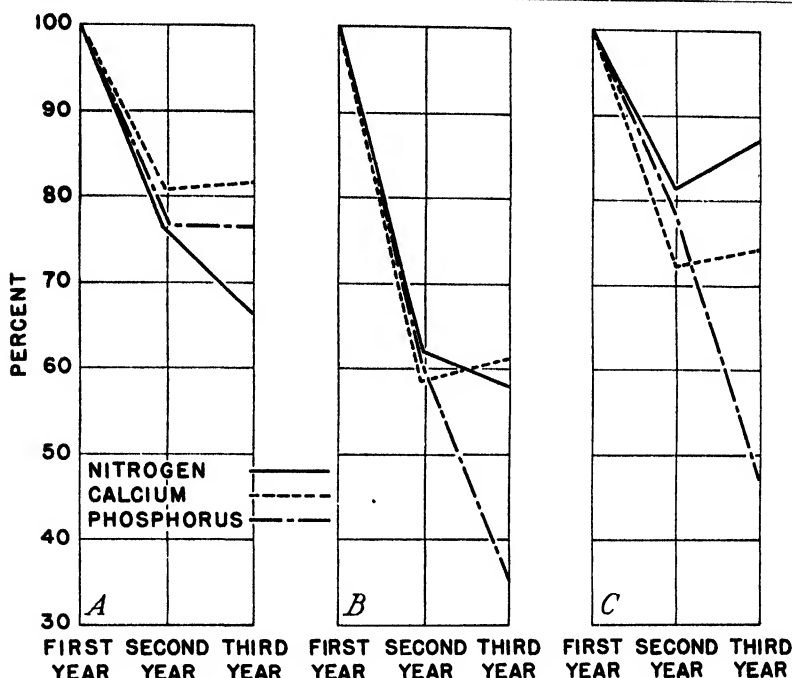


FIGURE 2.—Rate of decrease or increase in intake (A), retention (B), and percentage utilization (C) of nitrogen, calcium, and phosphorus by the high-phosphorus group of helpers as affected by increasing age.

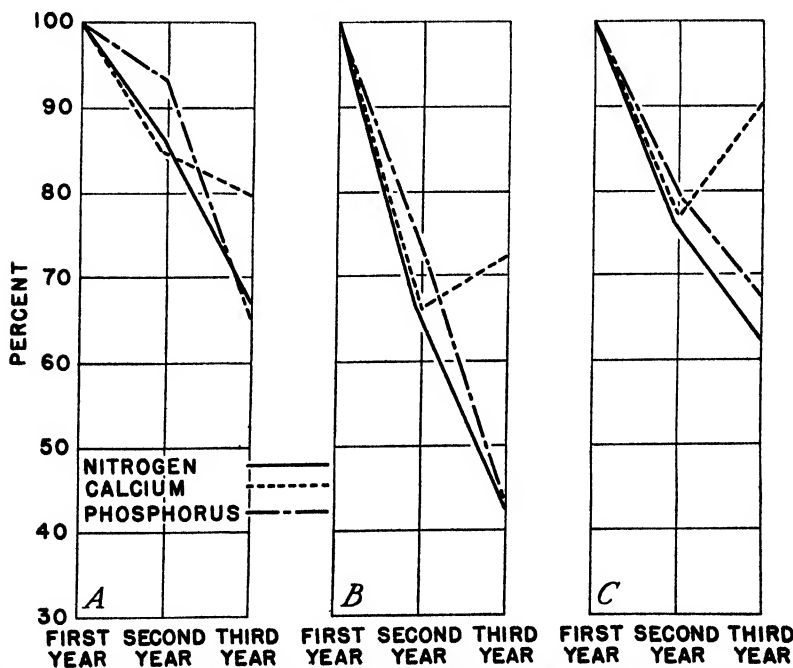


FIGURE 3.—Rate of decrease or increase in intake (A), retention (B), and percentage utilization (C) of nitrogen, calcium, and phosphorus by the low-phosphorus group of helpers as affected by increasing age.

rapid from the first year of life to the second than from the second to the third.

(2) That retention, per unit of weight, of all three elements decreased rapidly from the first year of life to the second, and, in the case of calcium, increased slightly from the second to the third year, the increase being more marked in the low-phosphorus group. Nitrogen and phosphorus retention continued to decrease in both groups during the third year.

(3) That in general percentage utilization (ratio of retention to intake), was roughly parallel to actual retention in its rate of decrease for both groups and the three elements.

The principal point of interest here is the upswing in retention and utilization of calcium in the third year for both groups, possibly a reflection of the demands of pregnancy.

CALCIUM PHOSPHORUS RATIO

Respecting ratio of calcium to phosphorus fed and retained no definite conclusions can be drawn. The tendency in retention has been toward a general level at around 1.5:1. The high-phosphorus group, with a somewhat lower average intake ratio, had an average retention ratio of 1:1.4, while the low-phosphorus group, with a considerably higher average intake ratio, leveled down on retention to 1:1.7. The exceptions to this statement are the retention ratios for 2-year-olds in both groups. It is thought that possibly the marked departures in these cases are due to the relatively small amount of data for that age group.

GROWTH AND REPRODUCTION RECORDS OF THE ANIMALS

The procedure for recording growth was the same as in the earlier work.¹ A graphic summary of the growth records appears in figure 4. Table 7 gives a record of the weight and condition at birth of each heifer's first calf.

TABLE 7.—*Weight and condition at birth of calves born to heifers in both phosphorus groups*¹

Calf of heifer no.—	High-phosphorus group		Calf of heifer no.	Low-phosphorus group	
	Weight at birth	Condition at birth		Weight at birth	Condition at birth
	<i>Pounds</i>			<i>Pounds</i>	
162.....	40	Good.	163.....	(⁴)	
175.....	55	Do.	168.....	(⁴)	
180.....	96	Do.	191.....	85	Good.
192.....	98	Excellent.	193.....	100	Excellent.
Average.....	96.3		Average.....	92.5	

¹ In all cases only one service was required for conception.

² Sired by a Jersey bull; not included in the average.

³ As previously noted, these animals had to be disposed of as yearlings.

⁴ No calf.

⁵ Retained the placenta.

The graphs reveal a quite uniform rate of growth. The low phosphorus group averaged smaller at the start and, with respect to girth,

¹ LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R. See footnote 3.

they continued so throughout the experiment. Their average weights were less until within a month before the experiment ended. Their superiority in height after the nineteenth month is attributed to the fact that data were available for only two heifers in this group (191 and 193) during the latter half of the experiment. This is confirmed by plotting the height curves for these two only for the entire experi-

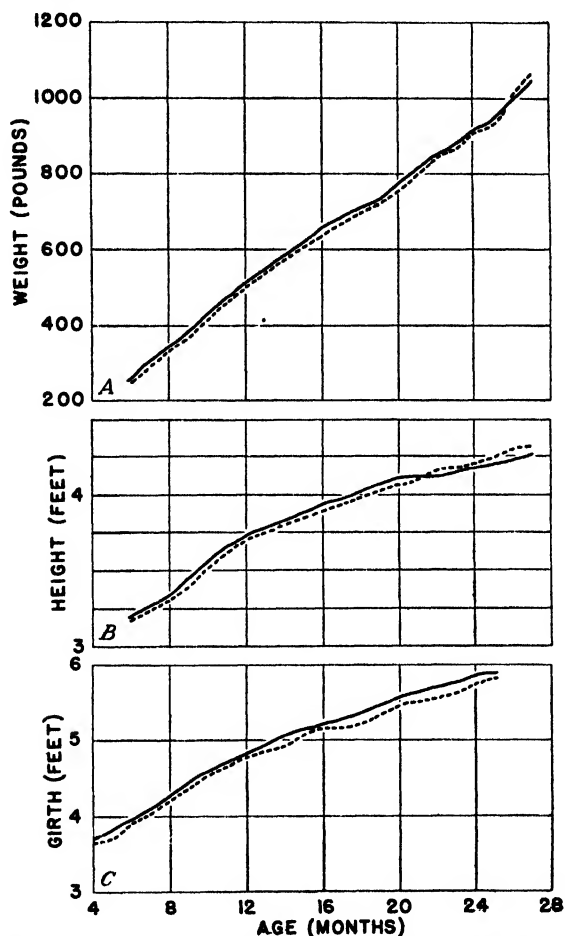


FIGURE 4.—Composite curves representing average weights (A), height at withers (B), and heart girths (C) of heifers in the high- and low-phosphorus groups. The solid lines represent the high-phosphorus and the broken lines the low-phosphorus groups. Commencing with the fourteenth month the curve for the low-phosphorus group is a composite for two animals only.

ment, and comparing them with the curves for their mates in the high-phosphorus group (190 and 192) also for the entire experiment. When thus segregated, the average height for the two in the low-phosphorus group is seen to have been greater throughout than that of their mates in the high-phosphorus group, indicating an obvious reason for change in the trend of the composite graph in the latter part of the period.

Differences in the evidence recorded in table 7 regarding the effect of the rations on the reproductive function are so slight that they are probably without significance.

In addition to this evidence secured while the heifers were on the experimental rations it is pertinent to include the fact that their subsequent history as cows with regard to either production or reproduction does not indicate that the high-phosphorus is superior in any way to the low-phosphorus group. If anything, the latter group has a more satisfactory record. It is possible, of course, that benefits from the larger amount of phosphorus stored by the high-phosphorus group may be apparent later on, that these individuals may have a longer productive life, or that they may be able to stand the strain of continued high production better than the low-phosphorus group.

DISCUSSION

From the data here presented it seems reasonable to conclude that heifers can make average growth on rations supplying amounts of phosphorus similar to those supplied by the low-phosphorus ration, that is, 1.8 g of phosphorus daily per 100 pounds of live weight during the first year of life, 1.7 g during the second year, and 1.2 g during the third year. That these amounts represent the optimum is not contended; probably they are somewhere close to the minimum. Hay containing less than 0.20 percent of phosphorus and consumed in normal amounts will not supply these amounts of phosphorus. Rowen and legume hays contain sufficiently more than that amount (0.20 percent) to provide a reasonable margin of safety even if nothing else is fed, but the average of a large number of analyses of ordinary mixed hays grown in Massachusetts shows only 0.16 percent of phosphorus, while some samples have been found that run as low as 0.10 percent. Where the roughage consists entirely of ordinary mixed hay, and especially where the quality is inferior, some other source of phosphorus should be supplied. The most logical and practicable way to make up possible deficiencies in this respect is through limited grain feeding, which is nothing more than most successful feeders do regularly.

The lowest phosphorus carrier among our common grains is corn, but even this will supply the deficiency when fed at a level of 3 pounds daily, while much smaller amounts of such high phosphorus carriers as wheat bran or linseed meal will supply the necessary amount.

SUMMARY AND CONCLUSIONS

In summarizing the results attention is called to the following points:

Retention and utilization of nitrogen and calcium were of a similar magnitude in both high- and low-phosphorus groups, such small differences as existed not being of statistical significance, except in the case of nitrogen storage by the 2-year-old subgroups. The apparently significant difference here may have been due, however, to an insufficient amount of data in these age groups.

Retention of phosphorus by the high-phosphorus group was superior at all ages, and significantly so during the first year. The low-phosphorus group made better use of the phosphorus they received, but not sufficiently so to equal the high-phosphorus group in phosphorus storage.

Differences in growth and reproductive function were very slight

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PRINCIPLES OF SNOW SURVEYING AS APPLIED TO FORECASTING STREAM FLOW¹

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SNOW SAMPLING

The purpose of snow sampling is to obtain an inelastic standard of measuring snow. The method of "accumulated snowfall", that is, the determination of total depth of newly fallen snow, storm by storm, necessarily involves errors due to variation in density of each snowfall. The average of many years will furnish a fairly accurate normal, but the seasonal measurement may be distorted. Rain gage measurements, even where the catch is reasonably accurate, do not represent the residue of snow or moisture available on March 1 or April 1. Snow stakes do not give the density at all, and even late in the winter after the snow has settled a new storm of fluffy snow may distort considerably the apparent water content of the snow.

APPARATUS:

A snow sampler, devised for use on Mount Rose, Nev., has been developed in strength and length to meet all conditions of mountain snow. The entire apparatus, even for the deepest snows, can easily be carried by two persons, and for medium snows can be carried comfortably by one. The basic principle of this sampler is a slotted tube ending in a spiral milled cutter which will cut snow cores however hard the snow may be. A slight offset or shoulder in the inside diameter of the cutter prevents the core from sliding down while being lifted. The slots permit the ready determination of the length of the core and facilitate the cleaning of the tube in case the snow adheres. The upper end of the tube is open, so that by the simple act of inverting, the snow core slides out quickly. Driving and clearing are accelerated by keeping the tube covered with a thin layer of shellac.

The length of the tube depends upon the usual depth of snow found. The measurements are effected by a scale of inches stamped along the length of the tube to measure the depth of the snow and the length of the core, and by a special spring balance by which the net inches of water in the snow sample can be read directly from the dial. Thus no computations are necessary in the field.

The notebook, treated by a special process to withstand snow and water,³ bears the columns for data essential to making up the forecast (fig. 1).

¹ Received for publication Dec. 10, 1934; issued September 1935.

² The description of the apparatus is taken in large part from the following publication: CHURCH, J. E. HUMBOLDT BASIN SNOW SURVEYS AND METEOROLOGICAL DATA. In Malone, G. W., Humboldt River Distribution and Different Features Affecting These Deliveries for the Years 1927 to 1931, Inclusive, ch. 5, pp. [87-103], illus. Carson City, Nev. 1932.

³ The use of waterproof paper for snow surveying was begun by the State of Oregon.

NEVADA COOPERATIVE SNOW SURVEYS

Reno, Nev.

SNOW SURVEY NOTES

Drainage basin _____
 Snow course { Name _____
 { Number _____ Spacing of samples _____
 Party chief _____ Date _____, 19____

Number of sample	Depth of snow, inches	Length of core, inches	Water con- tent, inches	Density $100 \times (4)/(2)$ percentage	Remarks
(1)	(2)	(3)	(4)	(5)	

N. B.—Sample no. 1 should not be taken at initial point or end of course but one space distant.

Number _____ of _____ sheets. Compiled by _____ Checked by _____

FIGURE 1.—Form of notebook page on which snow-survey data are recorded.

The average of the total measurements represents the measurement for the course. The density is determined by dividing the water content by the depth. In case too large a proportion of the cores are more than 2 inches short (a condition sometimes caused by sticking snow), the average density is computed only for those measurements where the cores are satisfactory, and this density is applied to the average depth of snow of all the measurements in the course. Thus errors due to the failure of snow to enter the sampler tube can be largely corrected. However, by resampling when the cores are short or by waiting for the snow field to freeze, most of the unsatisfactory measurements can be avoided.

Figure 2 shows the Mount Rose Observatory and the Tahoe Basin; figures 3 to 5 show the snow sampler in use in the field.

RELATIVE VALUE OF DIFFERENT METHODS OF FORECASTING SNOW SURVEYING

Two methods of forecasting based upon snow surveying have been developed—the percentage method and the method of areas. The former method is simple, being merely the determination of the percentage relationship of the seasonal snow cover of any given basin or region to its normal, the assumption being that such a percentage is indicative of the coming seasonal run-off in the streams below. By the latter method the surveyor endeavors to determine the acre-feet of water in the snow fields and the probable net amount that will find its way into the streams.

The difference between the two methods is fundamental. The percentage method is based upon the fact that the big storms which

furnish the bulk of the winter snow are comparatively uniform in intensity over considerable areas, thus lacking the spotted character of the lighter summer rains. Therefore, it is possible to select from

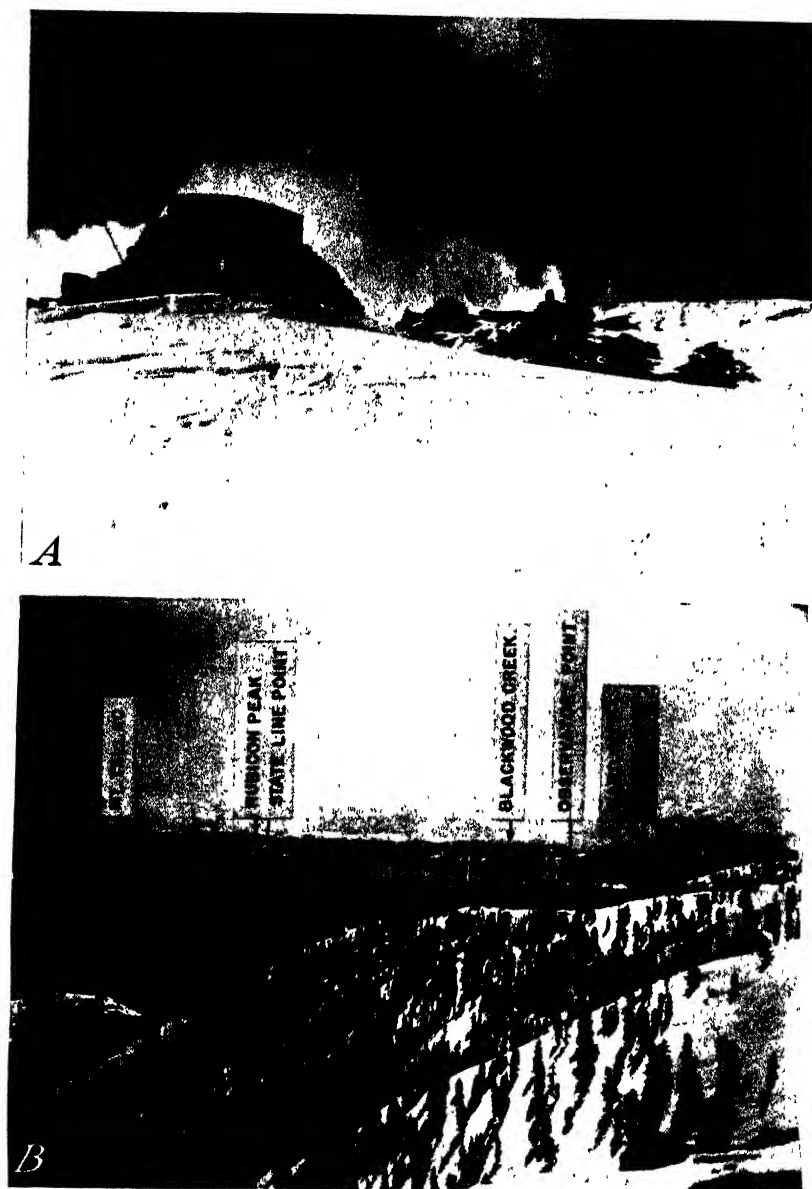


FIGURE 2.—A, Mount Rose Observatory at the crest of Mount Rose, elevation 10,800 feet; B, view of north and west sides of Tahoe Basin.

4 to 6 snow-survey courses distributed over characteristic parts of a basin and, by averaging their individual seasonal percentages, to obtain a close seasonal percentage for the basin as a whole. The only

requirements are that the snow courses shall be fixed and fairly long, such as 40 measurements 50 to 100 feet apart, and that they be located at a sufficient altitude to prevent distortion of the measurements by winter melting. A safe altitude on the western slope of the Sierra Nevada has been found to be approximately 7,000 feet. However, on the eastern slope and in the Humboldt Range, where the snow at this elevation is shallow, and therefore unstable, a higher elevation should, if possible, be sought. Sites for snow courses should be selected when possible, in protected flats or in timber, where the snow cover does not suffer oscillation from the wind.

The occurrence of early melting in the lower portions of the watershed and consequent distortion in the run-off indicated by the snow survey have caused the development of a zoning system. This



FIGURE 3.--Mount Rose snow sampler; the snow is as deep as the sampler is tall.

system consists of the division of the watershed into a series of altitude zones, measured by a planimeter on a topographic map, with a snow course and normal to represent each. The seasonal percentage for each zone is weighted according to the relative area of each. The average percentage of the zones represents the percentage for the watershed.

The method of areas is beset by two serious difficulties: (1) The large variation in the snow cover (even of individual basins) with elevation and distance from the crest of its watershed: (2) the difficulty of determining the normal seepage and evaporation losses of the region. The close agreement, when the precipitation during the period of run-off is fairly normal, between the snow cover and the run-off as determined by the percentage method is shown in table 1. The apparently wide divergence where the area method is used is shown in table 2.



FIGURE 4.—A, Mount Rose snow-sampler cutter; B, Mount Rose snow sampler being weighed with its core of snow.

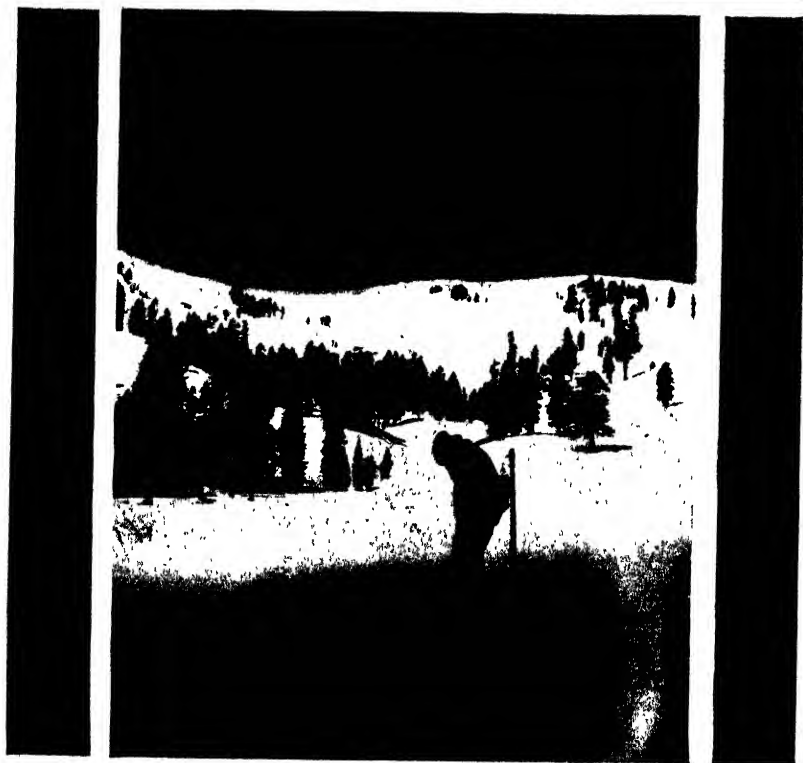


FIGURE 5.—Driving Mount Rose snow sampler (A) by hand and (B) by using the driving wrench and weight of the body.

TABLE 1.—*Similarity of snow cover in Tahoe Basin as determined by percentage method and rise of Lake Tahoe after Apr. 1, in years of approximately normal precipitation during run-off*

[Percentage of normal]

Item	1910-11	1911-12	1912-13	1913-14	1914-15	1916-17
Snow cover (average of 6 courses).....	170.4	49.7	58.2	153.8	88.2	115.6
Actual rise of lake after Apr. 1.....	172.3	64.5	69.3	160.6	89.8	125.9
Variation between snow cover and run-off.....	-1.9	-14.8	-11.1	+3.2	-1.6	-10.3

TABLE 2.—*Divergence between method of areas and percentage method of determining rise of Lake Tahoe after Apr. 1 in 1915 and 1916*

Year	Estimated rise by -				Actual rise		Divergence between estimated and actual rise as percentage of normal rise by—	
	Method of areas (corrected for normal losses by evaporation from snow and surface of lake and by absorption by the soil)		Percentage method (no corrections for normal losses necessary)					
	Feet	Percentage of normal	Feet	Percentage of normal	Feet	Percentage of normal	Method of areas	Percentage method
1915.....	0.08	5.1	1.46	88.2	1.49	89.8	-84.7	-1.6
1916.....	1.73	104.0	2.52	151.9	2.48	149.4	-45.4	+2.5

¹ Corrected by 50 percent for abnormal deficiency in precipitation during rise (see p. 105). Actual rise only 1.65 feet.

METHODS OTHER THAN SNOW SURVEYING

Three methods of forecasting based upon seasonal percentage but not upon snow surveying have entered or may enter into competition with the latter, because of their ease or inexpensiveness. These are the methods of accumulated snowfall, the winter rise of open lakes, and the depth of snow on the ground. The basis for choice between these methods and snow surveying is the degree of accuracy justified by expense.

ACCUMULATED SNOWFALL

An inherent weakness of the method of accumulated snowfall is the variation in the density of the snow, not only from storm to storm but even from season to season. This is particularly true where only the snow stake is employed and the water content of the snow cannot be determined because of distance of station from observer or because of the magnitude of the snowfall. In comparative measurements made in the Tahoe Basin by the Mount Rose Observatory and the United States Weather Bureau the divergence between this method and snow surveying varied between 20 and 55 percent for 5 seasons in a total of 8. Although this method is moderate in cost, its excessive latent inaccuracy makes it undesirable.

WINTER RISE OF OPEN LAKES

The winter rise of lakes (such as Lake Tahoe) which never freeze, should apparently be a safer standard for measuring the snowfall upon the adjacent watershed than the method of accumulated snow-

fall, as the snow falling upon the lake is reduced to water. The reverse is true, however, for the advantage of a fairly inelastic standard, such as water, is overcome by the variable factors of winter run-off from the watershed into the lake and the evaporation from its surface. In fact, a 9-year record shows that with a single exception the method of accumulated snowfall is from 5 to 40 percent more accurate.

DEPTH OF SNOW ON THE GROUND

Of the three methods that compete with snow surveying, the depth of snow on the ground at the beginning of the season of run-off is by far the most accurate, and if handled carefully is a close rival of snow surveying. The prime essentials are that courses equal in fixity and length to those used in snow surveying must be maintained and that the measurements of depth must be made only when the new snow has settled approximately to the density of the snow beneath. Fewer measurements, as at occasional snow stakes, or measurements immediately after a storm, would cause corresponding distortion in the results.

Whatever divergence occurs beyond the distortion due to the foregoing factors is caused by the seasonal density attained during the ripening of the snow. This density varies with the winter weather, particularly with temperature and wind, but the maximum variation between seasons is rarely greater than 10 percent. This is shown in table 3, which is based on snow surveys in the Tahoe Basin, in which both the depth of snow on the ground and the water content were determined over the same courses. Since the surveys were made without reference to the date of the previous storm the actual variation between the two methods should be less than that shown in the table.

TABLE 3.—Difference between depth of snow on the ground and water content of snow cover in the annual snow survey in Tahoe Basin, Apr. 1, 1909, to 1916

Method	Seasonal percentage for season indicated						
	1909-10	1910-11	1911-12	1912-13	1913-14	1914-15	1915-16
Depth of snow.....	80.0	165.1	59.4	66.5	141.9	93.6	145.5
Water content.....	82.7	170.4	49.7	58.2	153.8	88.2	151.9
Variation.....	-2.7	-5.3	+9.7	+8.3	-11.9	+5.4	-6.4

The gain over snow surveying is merely in saving the moderate expense for snow samplers. Aside from this the expense for field operations would be identical, without the satisfaction of certainty regarding the data obtained.

VARIABLE FACTORS AFFECTING RUN-OFF

Shrinkage and acceleration of run-off beyond normal occur occasionally, and must be taken into account in predicting the run-off or stream flow of an approaching season.

In forecasting the summer flow from winter snows the chief problem is to anticipate and predict the abnormal shrinkage that infrequently occurs in the seasonal percentage indicated by the April survey. In

the first 11 annual surveys made since 1909 in the Sierra Nevada this shrinkage has occurred to an extreme degree three times and to a moderate degree twice. The maximum shrinkage has been 50 percent of normal for lakes and 25 percent for rivers. Later computations indicate a maximum of 40 and 20 percent respectively.

PRECIPITATION DURING RUN-OFF

The major factor in the phenomenon of shrinkage in the predicted run-off is lack of precipitation during the period of run-off rather than late snowfall or dryness of the watershed. This is plainly indicated by the divergence between the maximum shrinkage in lakes and in rivers. Lakes are susceptible to precipitation in whatever amount, but streams, because of the preponderating land area of their basins, may be from 80 to 90 percent immune to such influence when the soil is driest. In fact, the disparity between the susceptible water areas of lake and river basins, as in the case of Lake Tahoe and the Carson River, may be as great as 50 to 1. When it is considered that evaporation is the complement of precipitation and intensifies its lack, the theoretical probability of apparently so small a cause as lack of late spring and summer rainfall producing so marked an effect on the run-off becomes more understandable. No other weather factors could have caused the divergence. For the Tahoe Basin this precipitation amounts normally to 22.8 percent of the snow cover. In April, when the snow cover is most extensive, it is 10.6 percent, and in May it is 9.5 percent additional.

Despite this fact an effectiveness of 25 percent seems at first anomalous in face of the further fact that occasional summer precipitation in the semiarid West is almost negligible as compared with a field of snow, because of the small proportion of the former that finds its way into the streams. However, at the time when the spring precipitation is heaviest the snow cover is most extensive, coming at times almost as low down as the gaging station, so that whatever precipitation falls at this period finds lodgment upon the snow surface and obtains a relatively untaxed passage to the streams.

As between one region and another, the amount of shrinkage in run-off depends upon the quantitative relationship of the precipitation during run-off to that of the period of accumulation and to the depth of the soil on the watershed. In the Sierra Nevada a deficiency of 100 percent in spring (i. e., April to July) precipitation seems to cause a shrinkage of 16.2 percent of normal in the run-off. However, in the Humboldt Basin in Nevada, where the spring precipitation is relatively four times heavier and the snow survey is made March 1, the shrinkage seems to be more than twice as great, or 36.1 percent. That it is not even greater is possibly due to the greater depth of soil.

In the case of lakes like Tahoe, where the lake surface bears a relationship of 2 : 3 to the land area of the watershed, the shrinkage in expected rise is double that in rivers. These estimates are subject to still further increase owing to increased evaporation attending lack of precipitation, especially when prolonged.

The relation of this fact to the April snow survey may be stated as follows: The equality between the seasonal percentage of the snow field, as measured April 1, and the succeeding run-off of April to July is dependent upon the normality of all factors that influence the run-

off, for the very normal upon which the seasonal percentage is based is merely the average of many seasons, and presumably of so many that any abnormality of the weather has faded into the general average.

MINOR FACTORS REDUCING RUN-OFF

Lack of autumn rains, snowfall less than normal, and shrinkage in run-off below diversion points are contributory but minor factors in the phenomenon of occasional shrinkage of run-off. The absence of autumn rains in the semiarid West seems to have little or no effect upon the amount of moisture necessary to prime the soil in the spring, and its effect even upon the winter run-off is not plainly discernible in the presence of other factors. Indeed, the effect of the heaviest snow covers in the Tahoe Basin, which have a cumulative effect far in excess of rain, does not persist beyond October, or 4 months beyond its peak. This is due to the steep mountain slopes and a surface material sufficiently porous to permit a rapid run-off. Meadows and other retarding media are small in area as compared with the entire watershed, so that even the heavier rains are drained off before the winter has arrived. Moreover, the freezing of the surface soil, so effective on priming in the East, is entirely lacking, for the surface has been drained dry before the freezing temperatures of winter occur.

The effect of subnormal snowfall in reducing the computed seasonal percentage of run-off may be obscured by excess or deficiency of precipitation during run-off, but it can be determined mathematically if the initial factors are known. Since the amount of moisture necessary to prime mountain soil in semiarid regions is practically unaffected by weather, and hence may be considered a fixed quantity for its particular basin, it becomes a factor of increasing importance as the snow cover falls below normal. For example, the normal snow cover of the Tahoe Basin estimated quantitatively by the method of areas is 22.7 inches water content and the amount of absorption in priming the soil theoretically determined is 8.6 inches, or 37.9 percent. This factor of absorption was obtained in the following manner. The actual mean rise of the lake to April 1 was 1.46 feet, and the mean rise after April 1 was 1.66 feet. Since the rise to April 1 is due largely to precipitation on the surface of the lake, it is safe to assume a precipitation fully as great, and apparently 1.76 times greater on the land area that drains into it, judging from the relation of the precipitation around the edge of the lake to the average precipitation over the slopes above it (based on the relationship of 176.1 percent between the snow at Ward Creek, elevation 7,000 feet, and Tahoe city). Furthermore, the land area is 1.5 times larger than the water surface, or, more exactly, 306.9 square miles to 192.7 square miles, thus making a total preponderance of 2.6 times. Therefore, if there were no accretions from the watershed and no losses through evaporation, the absorption would be determined thus:

$(1.76 \times 306.9) : 192.7 :: \text{estimated mean rise} : 1.46 \text{ feet.}$

Estimated mean rise after April 1 due to melting snow

(uncorrected for absorption)..... 4.09 feet.

Actual mean rise..... 1.66 feet.

Mean absorption..... 2.43 feet=59.4 percent.

However, on the basis of the flow of the neighboring West Fork of the Carson River above 5,600 feet, approximately 15 percent of the rise before April 1 can be attributed to run-off during the winter from the watershed, leaving 1.24 feet as the rise due to direct precipitation on the lake. On the other hand, the winter run-off causes some loss of snow on the watershed. At the ratio of 2.64 (relative precipitation and area) to 1, this would amount, if distributed over the watershed, to 5.7 percent, which should be still further decreased because the winter run-off is fed in part by autumn rains. Consequently, this loss may be regarded as negligible.

Again, since the water of the lake evaporates more rapidly than the snow on the watershed, a further correction should be applied to the lake level to restore the balance. For this an approximate factor of 3.5 inches or 0.29 foot is proposed for the period preceding April 1, and the same or a slightly larger factor for the period following it.

The actual mean rise of the lake to April 1, as thus corrected for difference in evaporation, would be 1.53 feet ($=1.24+0.29$), and the rise or run-off after April 1, 1.95 feet ($=1.66+0.29$). The absorption would then appear thus:

$(1.76 \times 306.9) : 192.7 :: \text{estimated mean rise} : 1.53 \text{ feet.}$

Estimated mean rise (corrected for difference in evaporation but uncorrected for absorption).....	4.29 feet.
Mean actual rise (corrected for evaporation).....	1.95 feet.
Mean absorption.....	2.34 feet = 54.5 percent.

The quantitative determination of the absorption is more difficult because of the lack of a quantitative normal for the watershed. However, the unexpectedly close agreement between the trial normals for 1914-15 and 1915-16, the years under discussion, computed on the basis of the individual seasonal percentage of each, encourages their adoption as a satisfactory joint substitute. Table 4 gives the details.

TABLE 4.—*Forming a quantitative normal from seasonal percentage*

Year	Average water content of snow cover	Seasonal percentage of snow cover	Quantitative normal for snow cover
	<i>Inches</i>		<i>Inches</i>
1914-15.....	19 93	88.2	22.60
1915-16.....	34 66	151.9	22.81
Quantitative normal of snow field.....			22.71

On the basis of the foregoing quantitative normal, the mean quantitative absorption, 54.5 percent of the snow field, amounts to 12.38 inches. However, since one-third of this amount has already entered the soil, especially on the lower levels, by April 1, when the survey is made, the net mean absorption is estimated at only two-thirds of the foregoing amount, or 8.25 inches.

The decrease in run-off with decrease in seasonal percentage of snow for the Tahoe Basin is shown in table 5.

TABLE 5.—*Decrease in run-off with decrease in seasonal percentage of snow for Tahoe Basin*

Snow cover		Absorption	Run-off		Decrease from snow cover of normal run-off
Percent	Inches		Inches	Percent	Percent
100 ¹	22 7	8 6	14 1	100	. 0
75.....	17 0	8 6	8 4	59.5	15.5
50.....	11 4	8 6	2 8	19.9	30.1
25.....	5 7	8 6	2 -2 9	20.5	45.5

¹ Normal² The minus sign indicates the entire absence of run-off, and the figures 2 9, 20.5, and 45.5 indicate the amount of deficiency.

Shrinkage in the expected flow below diversions is similar in effect to subnormal snow, for not only is the ratio of decrease progressive, but the land is thirstier because of the general dryness of the season. In years of heavy precipitation the diversions tend to increase the seasonal percentage, as based upon the snow fields. One of the observations made in 1919-20 is shown in Table 6.

TABLE 6.—*Shrinkage in expected run-off below diversions for Carson Basin with Tahoe and West Walker Basins as checks; April-July 1920*

Basin	Expected run-off			Diver- sions	Actual run-off		Dimi- nution from normal
	Normal	Seasonal 1919-20			Amount	Percent- age of normal	
	<i>Acre-feet</i>	<i>Percent</i>	<i>Acre-feet</i>	<i>Acres</i>	<i>Acre-feet</i>		<i>Percent</i>
Carson (Empire) -----	246,412	70.0	172,488	27,000	97,496	39 6	30 4
Tahoe -----	1 1 66	50.2	1 0 83	None	1 0 89	53 6	3 4
West Walker -----	190,366	74 8	142,394	Small	164,100	66 2	11 4

¹ Rise.

This shrinkage is shown conspicuously in the variation in the ratio of the use of water in the Truckee Meadows with variations above and below normal in the stream. These meadows consist of approximately 35,000 acres of irrigated and swamp land around Reno, Nev., supplied by a normal April to July run-off of 350,000 acre-feet. During the 12 years from 1908 to 1919, the average variation at the gaging stations above and below all diversions in the Truckee River, which is the main source of supply, was as follows: (1) With run-off above normal, +8,676 acre-feet; (2) at normal, -2,668 acre-feet; (3) below normal, -55,760 acre-feet. In terms of the normal run-off of this river the percentage of variation is +2.7 when the stream is above normal; -0.8 percent when at normal; and -17.1 percent when below normal. The data are shown in table 7.⁴

⁴ NORCROSS, C. A. WATER COST OF IRRIGATION, TRUCKEE VALLEY, NEVADA. Nev. Agr. Col. Ext. Bull. 25:16. 1919.

TABLE 7.—*Effect of diversions in Truckee Basin, Nev., April-July*[Normal run-offs, 325,745 acre-feet ¹]

SEASONS ABOVE NORMAL RUN-OFF

Year	Snow cover percentage of normal	Run-off in acre-feet			
		At State line (above diversions)	At Vista (below diversions)	Average variations	
				Amount	Percent- age of normal
	Percent (²)	Acre-feet	Acre-feet	Acre-feet	
1908-9		600, 200	582, 900		
1910-11	176.4	789, 000	799, 000		
1913-14	142.1	544, 442	622, 540		
1915-16	148.9	473, 850	452, 780		
1916-17	118.9	449, 510	443, 160		
Average		571, 400	580, 075	+8, 676	+2.7

SEASONS OF NORMAL RUN-OFF

1909-10	76.7	308, 400	337, 700		
1914-15	83.2	341, 650	361, 870		
1918-19	88.1	349, 818	292, 294		
Average		333, 289	330, 621	-2, 668	-0.8

SEASONS BELOW NORMAL RUN-OFF

1907-8	(²)	231, 800	164, 900		
1911-12	48.5	200, 400	136, 300		
1912-13	52.9	215, 590	175, 700		
1917-18	89.6	253, 548	201, 400		
Average		225, 335	169, 575	-55, 760	-17.1

¹ Additional run-off from creeks flowing directly into the Truckee Meadows 22,000 acre-feet (estimated).² Previous to establishment of snow survey.³ Though snow cover was nearly normal, the precipitation during run-off was deficient 63 percent.

The failure of the ratio to rise in seasons above normal as much as it falls in seasons below normal is evidently due to the relatively small draft made by the meadows upon the waters of the Truckee and auxiliary creeks, even when the stream is only at normal. Discrepancies in individual seasons, as in 1918-19, are rare and may be due to other factors, such as previous condition of soil moisture or variations in rate of evaporation.

Most of the factors mentioned in the foregoing discussion are illustrated in table 8, based upon 4 seasons of deficient precipitation during run-off, with 6 check seasons of normal spring and summer rainfall. A lake and a river basin adjoining each other, and consequently exposed to similar weather conditions, are selected to show the divergence between the types.

The dominant and consistent correlation is the precipitation, the river basin being less affected by extremes than the lake basin. The priming of the soil by autumn rains or winter run-off presents constant contradictions. The effect of the extremely subnormal years of 1911-12 and 1912-13 is obscured by excess precipitation, and shrinkage below diversions during these years does not appear, possibly also because of the summer rainfall. The data in this table are con-

firmed by those obtained in the season of 1919-20, immediately following, in which the forecast for was approximately 20 percent below normal, and the summer rains, except for two heavy showers, were entirely lacking. The diminution in run-off seems to have been as high as 30 percent.

TABLE 8.—Effect of weather upon seasonal run-off in seasons with and without abnormal shrinkage in run-off in Carson River and Tahoe Lake Basins.

SEASONS OF ABNORMAL SHRINKAGE IN RUN-OFF (PERCENTAGE OF NORMAL)
CARSON RIVER BASIN

Year	Water content, of snow cover	Priming of soil as shown by excess and deficiency of autumn rains and winter run-off.		Excess and deficiency of precipitation and evaporation.		Run-off	
		Autumn rains	Winter run-off	Precipitation	Evaporation	Seasonal percentage	Variation between snow cover and run-off
1909-10.....	182.7	+165.41	+38.31	-29.2	-----	64.14	-18.6
1915-16.....	1151.9	-69.55	+49.59	-70.4	-----	125.71	-26.2
1917-18.....	96.4	-25.19	-29.51	-57.2	² +27.7	³ 61.31	³ -35.1
1918-19.....	96.8	+100.00	-44.52	-0.8	² +36.2	66.58	-30.2

SEASONS OF ABNORMAL SHRINKAGE IN RUN-OFF (PERCENTAGE OF NORMAL)
TAHOE LAKE BASIN

1909-10.....	182.7	+140.18	⁴ +38.31	-86.9	-----	61.45	-21.2
1915-16.....	151.9	-42.83	⁴ +49.59	-82.9	-----	99.40	-52.5
1917-18.....	95.0	-71.96	⁴ -29.51	-63.0	+27.7	53.61	-41.4
1918-19.....	103.0	+113.69	⁴ -44.52	-63.5	+36.2	⁴ 72.89	⁵ -30.1

SEASONS WITHOUT ABNORMAL SHRINKAGE IN RUN-OFF (PERCENTAGE OF NORMAL)
CARSON RIVER BASIN

1910-11.....	1170.4	-18.80	+7.36	+26.8	-----	176.70	+6.3
1911-12.....	149.7	-40.98	-40.75	+51.0	-----	42.43	-7.3
1912-13.....	154.2	+22.18	-57.30	+72.4	-----	57.18	-1.0
1913-14.....	1153.8	-16.92	+81.89	+3.5	-----	162.95	+9.2
1914-15.....	188.2	-48.50	-17.35	-4	-----	93.33	+5.1
1916-17.....	1115.6	+30.08	+19.08	+4	² +6.0	128.65	+13.1

SEASONS WITHOUT ABNORMAL SHRINKAGE IN RUN-OFF (PERCENTAGE OF NORMAL)
TAHOE LAKE BASIN

1910-11.....	170.4	-12.80	⁴ +7.36	+3.27	-----	172.29	+1.9
1911-12.....	49.7	-60.26	⁴ -40.75	+63.48	-----	64.46	+14.8
1912-13.....	58.2	+27.15	⁴ -57.30	+12.59	-----	69.28	+11.1
1913-14.....	163.8	-6.80	⁴ +81.89	-4.53	-----	150.60	-3.2
1914-15.....	88.2	-56.73	⁴ -17.35	+11.84	-----	89.76	+1.6
1916-17.....	115.6	-13.02	⁴ +19.08	-23.43	² +6.0	125.90	+10.3

¹ Substituted from Tahoe Basin because of lack of snow surveys.

² From floating pan at Tahoe City.

³ At Clifton, 20 miles below Empire. Probable correction 5 percent; seasonal 66.31 and variation 30.1.

⁴ Interpolated from Carson River.

⁵ Maximum rise accelerated 3 weeks by high temperature. If corrected for evaporation to normal date of maximum elevation, July 7, seasonal percentage of run-off would be 60.89 and variation 42.1.

APRIL PRECIPITATION

The precipitation factor in table 8 is made more obvious by the preponderance of the April or April-May precipitation over the remaining precipitation in the seasons of 1913-14, 1914-15, and 1916-17. Thus, in the Tahoe Basin in 1913-14, nearly two-thirds of the total precipitation during run-off fell in April; in 1916-17, more than

two-thirds; and in 1914-15, although only 0.54 inch fell in April, this was supplemented in May by 3.70 inches, the joint precipitation amounting to ninety-five one hundredths of the total for the run-off period.

In the Tahoe Basin the normal April precipitation (1.78 inches) is estimated at 10.6 percent and the May precipitation (1.60 inches) at 9.5 percent of the normal winter snow cover, while the precipitation for June is only 2.7 percent and that for July 1 to 15 is negligible. Thus, in view of the continuous melting of the snow fields after March 15 and the increasing area of exposed soil, the precipitation for April should indicate the probability of normality or shrinkage in the seasonal run-off. It is possible, however, that the May precipitation at times may become the deciding factor, or that concentrated rain, as in 1919-20 may complete the monthly or seasonal normal and leave bright skies to accelerate the evaporation. But this latter effect is only partial as compared with total lack of rain.

ACCELERATION OF RUN-OFF

Acceleration or even retardation of snow melting does not necessarily mean abnormal increase or decrease in the relative seasonal run-off, for the melting when once begun is almost continuous. Consequently, the soil has no opportunity to dry out, and the total variation in run-off is probably less than 8 percent.

It is a striking fact that in all of the seasons of abnormal shrinkage in run-off shown in table 8 the seasonal temperature during run-off was from 0.7° to 4.2° F. in excess of normal. This temperature was 2.5°, 0.7°, 3.0°, and 4.2° F. in excess for the seasons of 1909-10, 1915-16, 1917-18, and 1918-19, respectively. Its principal effect was to run the moisture from the snow fields prematurely, without noticeably increasing the total seasonal flow. The percentage of normal run-off in the Carson Basin in 1918-19 was as follows: April, 86.4; May, 115.2; June, 27.6; July, 4; April-July, 58.3. The excess in temperature at Reno, Nev., during April was 2.1°; May, 7.2°; June, 1.8°; July, 5.7°; April-July, 4.2° F. The percentage of normal snow cover on April 1 was 98.6 and the variation between run-off and snow cover 30.2 percent below normal.

Although the May flow is 16.6 percent above that of the snow cover, the June flow falls to a deficiency of 71 percent, while the July flow is negligible. The total seasonal loss of 30.2 percent, due to lack of rains, is so large that little compensation can have come from the accelerated melting, but it is possible that the increased ratio of shrinkage below diversions accompanying subnormal run-off may have clouded the result.

LIGHT DENSITY OF LATE SNOWS

That late mountain snows have a tendency to run off prematurely is not proved by data so far gathered. The fact is rather that these snows pass through a process of accelerated ripening as a result of the alternation of thawing and freezing that occurs normally in April above the elevation of 7,000 feet. For example, at Summit Station, Calif., in 1917-18, the relative density of the snow field for its entire depth was only 36.6 percent on March 24, but on April 15 it had increased to 49.2 percent with a loss of only 3.3 inches above the current precipitation in a total snow cover of 32.8 inches.

It is true that snow of light density at the beginning of the time of melting, before losing its water, fails to attain the density of snow of higher initial density. But the maximum seasonal variation in density for the Tahoe Basin as a whole is under 13 percent, and no marked effect from light densities appears in the run-off. This is shown in table 9 by the lack of correlation between deficiency in density⁵ and the advancement of the month of maximum run-off or even of acceleration in the melting, which latter is due mainly to excess of temperature.

TABLE 9.—*Lack of correlation between light density of snow cover and early maximum run-off in Carson River Basin, 1909-10 to 1916-16*

Year	Snow cover in Tahoe Basin (percentage of normal)	Excess or deficiency in density of snow	Excess or deficiency in temperature from March to month of maximum acceleration inclusive	Month of maximum run-off (normal month, May)	Percentage of monthly normal run-off to show acceleration ¹					
					March	April	May	June	July	August
		<i>Percent</i>	<i>° F</i>							
1909-10.....	83.3	+3.3	+7.5	May.....	134.43	127.66	74.54	34.18	19.01	-----
1910-11.....	167.5	+2.4	+7	June.....	142.01	135.63	113.05	236.24	258.36	161.10
1911-12.....	49.9	-9.5	-1.2	do.....	37.0	7.62	52.78	61.83	12.61	-----
1912-13.....	58.3	-8.2	+6	May.....	44.35	57.09	92.35	36.26	14.0	-----
1913-14.....	154.1	+12.2	+4.2	do.....	162.10	163.29	178.65	148.95	156.06	141.37
1914-15.....	88.4	-5.2	+2.0	June.....	71.40	95.23	83.11	108.69	77.16	-----
1915-16.....	152.2	+6.7	+4.4	May.....	134.39	161.95	121.58	118.52	101.40	-----

¹ Maximum accelerations shown in bold-faced type.

CHARACTER OF STREAM BED

An exception to the foregoing statements must be made in the case of the Humboldt Basin. Owing to the alluvial character of the stream bed and obstructions in the channel, an unusual amount of water is required for priming the river. This amount becomes excessive when the stream bed has become dry because of a preceding dry year. As high as 20 percent of the normal run-off has been required for such priming, and in 1931-32 a correction of 30 percent was made for superdryness resulting from a succession of dry years. In the Humboldt Basin this factor is next in importance to precipitation during run-off.

PREDICTING RUN-OFF FROM LARGE AREAS

UNIFORMITY IN THE SNOW COVER

Uniformity of the snow cover in adjoining basins is an important feature in making practicable the forecasting of run-off from large areas. This condition of uniformity is due to the fact that winter storms which build the snow cover on the mountains are usually wide-spread in their sweep and that the gradient of their intensity is

⁵ See column 3, table 9.

low and uniform for relatively wide distances. This is particularly true of the "big storms" that often furnish the bulk of the winter precipitation. However, in seasons of heaviest snow cover, the oscillation or variation between basins (as shown by river flow in table 10) appears at times, but not uniformly, to be more accentuated than in seasons of normal or light snow cover.

The similarity of snow cover in adjoining basins is indicated distinctly by the snow surveys of 1917-20, as shown in table 10; yet the last two basins, Carson and Walker, cover a joint distance of 95 miles.

The gradient of seasonal percentage increased toward the south in 1917-18 and 1919-20, but toward the north in the intervening year. This uniformity is not a local peculiarity, but has been found also in the Wasatch Range, Utah. For example, the snow surveys of 1913-16 in the City Canyon and Big Cottonwood Canyon Creeks, conducted by the United States Weather Bureau and the engineering department of Salt Lake City, respectively, varied from each other during the entire period only 1.7 and 2.9 in seasonal percentage. The distance between these basins is 14 miles.

TABLE 10.—*Similarity of seasonal percentage of snow cover in adjoining basins 1917-18 to 1919-20*

Season	Percentage of snow cover in indicated basins, each being represented by 1 central station			
	Yuba (Summit station)	Tahoe (Rubicon Peak)	Carson (Blue Lakes)	Walker (Center Mountain)
1917-18.	82.5	90.6	96.4	-----
1918-19.	107.1	195.5	96.8	97.0
1919-20.	71.6	73.3	75.3	88.2

¹ The average of the 3 crest stations, Ward Creek, Rubicon Peak, and Lake Lucile, is 104.7

CONDITIONS IN THE SIERRA NEVADA RANGE

To extend the study of uniformity of the snow cover beyond the present limit of the snow surveys, provisional recourse has been had to the run-off of such streams in the Sierra Nevada as were obviously dependent upon the snow cover. To reduce the streams to a common standard, the seasonal percentage of each in terms of its own normal was taken. Furthermore, to avoid all possible influences except that of the snow, the normals and seasonal percentages were confined to April-July. Finally, the streams were grouped laterally and transversely along the range to determine the probable amount of oscillation in the snow and the detailed snow surveys necessary to detect it.

Table 11 includes particularly the division by slopes. The western slope is fully represented from the Klamath to the Kern. The eastern slope still lacks the minor basins of Honey Lake and Goose Lake in the north, for which no data are available. The foothill streams, being fed only to a minor extent by snow, are segregated from the rest. The primary peak of their flow occurs in midwinter, and only occasionally does their April-July run-off correspond closely with that of the crest streams.

If the run-off of the 15 crest streams on the western slope were represented graphically, there would be a curve varying from end to end annually from 24.9 to 299.4 percent. The latter represents the season of 1915-16, when the apparent snow cover increased from 98.3 percent in the upper Klamath Basin at the northern end of the range to 390.3 percent in the Kern Basin at the far southern end, and disastrous floods occurred even at San Diego.

If the slope is divided into three sections of approximately 150 miles each, the extreme annual variation in each would be somewhat reduced: Northern Sierra section, 8.6 to 91.4 percent; central Sierra 11.1 to 49.4 percent; southern Sierra, 9.7 to 259.0 percent, the latter occurring the year of the flood. If the upper Sacramento-McCloud and Kern Basins, situated at the extreme ends of the range, are treated as parts of new sections, the variations in the plateau section should be reduced to the extremes of 6.1 and 27.4 percent and in the southern Sierra sections to 2 and 57.9 percent. Finally, and of greatest importance for forecasting, the individual percentages for several adjoining basins represent, with occasional exceptions, a fairly uniform curve of change, not only within the several sections but also overlapping from one section to another.

Even closer uniformity exists on the eastern slope, if the rise of Lake Tahoe is corrected for excess and deficiency of precipitation upon its surface to make the run-off in its lake basin more comparable to that in river basins. The variation might be even smaller if corrections were made for evaporation and diversion of water in the Tahoe and Carson Basins respectively.

Likewise, if a cross section of the two slopes is taken, a similar uniformity in the snow fields, with occasional variation, is shown by the streams that head in a common summit but flow down the range in opposite directions. Unfortunately, all examples are confined as yet to the northern Sierra section. In the Truckee-Yuba Basins the extremes in annual variation are 3.7 and 36.1 percent; in the Tahoe-American 0.3 and 42.4 percent; in the Carson-Mokelumne Basins, 2.3 and 49.2 percent; and in the West Walker-Tuolumne Basins, 0.1 and 20.1 percent. In this last case, data are lacking for the year of probably maximum variation. Table 12 furnishes details of the records.

TABLE 11.—*Variations in percentage of normal run-off, April-July period, 1909-10 to 1918-19 between streams¹ on the same slope of the Sierra Nevada*

Side and section of range and total length	Basin	1909-10	1910-11	1911-12	1912-13	1913-14	1914-15	1915-16	1916-17	1917-18	1918-19
East side:											
Central Sierra (110 miles).....	Tahoe ¹	73.85	172.29	55.76	67.68	151.60	88.76	111.20	127.40	62.61	81.99
	Carson.....	64.14	176.70	42.43	57.18	162.95	93.53	125.71	128.65	61.31	66.58
	West Walker.....	96.57	150.98	50.23	50.96	162.95	93.53	119.98	106.85	81.86	70.34
	Maximum variation.....	32.4	98.1	13.8	16.7	11.4	4.6	14.5	21.8	20.6	18.4
Southern Sierra (65 miles to gaging station).....	Owens.....	92.8	141.8	61.5	61.9	143.7	86.0	99.3	117.5	73.1	73.1
Maximum variation of east side.....		32.4	34.9	19.1	16.7	19.5	7.3	28.4	21.5	28.6	18.4
West side (66 miles):											
Northern Sierra (approximately 120 miles).....	Sacramento.....	71.0	112.2	80.2	79.0	115.8	141.3	81.5	91.0	53.0	69.8
	Feather.....	60.4	153.7	46.2	68.2	123.7	139.8	143.7	114.1	47.0	72.8
	Maximum variation.....	10.4	71.5	34.6	8.9	12.7	11.7	14.9	28.1	6.0	9.0
	Tuba.....	60.6	146.10	56.56	68.0	112.61	117.97	111.87	104.93	47.67	70.82
	American.....	77.88	184.25	53.20	61.13	109.25	124.04	110.88	110.40	53.46	70.85
	Mocketunne.....	79.33	168.32	53.44	61.13	109.25	109.01	106.88	110.88	53.46	70.85
Central Sierra (approximately 210 miles).....	Stanislaus.....	76.39	163.62	49.80	44.14	103.75	105.46	116.82	109.74	69.65	73.29
	Maximum variation.....	18.3	130.66	59.82	59.49	113.17	105.65	102.33	107.05	73.12	67.21
	Merced.....	82.4	176.1	54.0	47.7	118.7	18.6	6.5	8.0	26.5	11.8
	San Joaquin.....	78.8	142.3	53.8	49.7	128.9	90.1	127.4	102.0	77.7	71.2
	Kings.....	50.0	116.4	53.1	49.7	128.8	101.7	150.1	97.5	76.4	60.2
	Kaweah.....	87.6	174.1	62.5	53.9	98.3	95.8	151.3	105.8	55.1	68.0
	Kern.....	37.6	57.9	8.7	11.3	70.3	113.0	390.3	130.1	83.1	78.5
	Maximum variation.....	32.4	57.9	2.0	11.3	38.6	8.4	299.0	41.9	58.0	18.4
	Maximum variation exclusive of Kern.....	37.6	71.9	34.0	38.3	72.0	45.5	177.5	48.1	38.1	16.8
Foot-hill streams, west side.											
Central Sierra.....	Cosumnes.....	77.23	218.65	62.88	57.24	91.34	153.42	114.48	142.02	64.72	72.0
Southern Sierra.....	Tule.....	58.7	112.8	63.2	37.7	95.5	154.3	207.3	147.8	36.2	65.8

¹ These streams are also assembled in groups and the maximum variation within each group is given, likewise the maximum variation for the slope as a whole.
² The rise of Tahoe has been corrected for excess or deficiency of precipitation upon its surface to make its rise more comparable with the run-off of the streams.
³ At Clifton, 21 miles below Empire, where measurements are usually taken. Correction for diversion should probably be +5 percent.

TABLE 12.—*Variation between run-off of streams on opposite slopes of Sierra Nevada that have a common summit, for April-July periods, 1909-10 to 1918-19*

Section	Basin	Percentage of normal run-off for years indicated									
		1909-10	1910-11	1911-12	1912-13	1913-14	1914-15	1915-16	1916-17	1917-18	1918-19
North	Tahoe ¹	73.85	172.29	55.70	67.68	151.60	88.76	111.20	127.40	62.61	81.99
	Yuba.....	66.67	146.10	56.58	68.10	112.61	117.27	111.87	104.93	47.67	70.82
	American.....	77.58	184.12	56.20	60.13	109.25	124.04	110.88	110.10	53.46	76.85
	Maximum variation ²	7.3	26.2	.8	7.6	42.4	35.3	.3	22.6	14.9	11.2
Middle	Carson.....	64.14	176.70	42.43	57.18	162.95	93.33	125.71	128.65	61.31	66.58
	Mokelumne.....	79.35	168.22	53.24	61.84	113.75	109.01	123.28	113.54	63.64	75.29
	Stanislaus.....	74.31	163.92	49.80	44.14	105.16	105.46	110.32	109.74	60.65	61.09
	Maximum variation ²	15.8	12.8	10.8	13.0	57.8	15.7	15.4	18.9	2.3	14.2
South	West Walker.....	96.57	150.58	56.23	50.96	---	---	119.98	106.85	81.95	70.34
	Tuolumne.....	76.49	150.66	59.82	59.49	113.17	105.65	102.33	107.95	73.12	67.21
	Maximum variation ²	20.1	.1	3.6	8.6	---	---	6.7	1.1	8.8	3.1

¹ Corrected for rainfall on surface of the lake.² Bold-faced figures indicate variation between slopes³ At Clifton, 20 miles below Empire, where measurements are usually taken; correction for diversion should probably be +5.0 percent.

OSCILLATION IN THE SNOW COVER

The second marked characteristic of the snow cover, as shown by the flow of the streams (table 11), is its occasional heavy oscillation along or across the range. A striking illustration of the former is the continuous and frequent zigzag in the seasonal percentage between the basins along the western side of the Sierra Nevada proper in 1910-11, when the maximum variation even within the individual sections was at its peak of 38.0 to 57.9 percent. Another illustration is the sudden upward sweep in the Kern Basin of 72 percent from the neighboring Kaweah Basin in 1913-14 and 239 percent in 1915-16.

Oscillation across the range is illustrated by the diversity in the stream flow in the northern Sierra section in 1913-14 (table 12). Although the maximum oscillation along the range, as measured on the western side, was only 18.3 percent, the oscillation across the axis in the four several groups of basins varied from 28.5 to 49.2 percent, the excess being uniformly on the eastern side.

QUADRANGLES IN FORECASTING

On the basis of stream flow, both uniformity and oscillation over the range can be detected and forecasted most accurately by dividing the range into a series of sections or quadrangles, the length of each according, theoretically, with the length of oscillation (fig. 6). But, practically, the quadrangle should be short enough to keep the normal oscillation within reasonable limits without regard to any overstepping from one section to the other. The width of the quadrangle should be sufficient to include all foothills above the elevation of 6,000 to 8,000 feet on both sides of the crest, in order to furnish as long a transverse base line as possible. Crest stations with outposts on both sides should be maintained to reveal any deviation in the seasonal percentage either along or across the range. Each basin should be represented by one or more crest stations, the number depending upon the size of the basin or the reliability of the station.

In the original California-Nevada cooperative snow survey, three quadrangles were tentatively formed. The oldest was the central Sierra quadrangle, comprising the Honey, Tahoe, Carson, and Walker Basins on the eastern slope, and the Feather, Yuba, American, Moke-

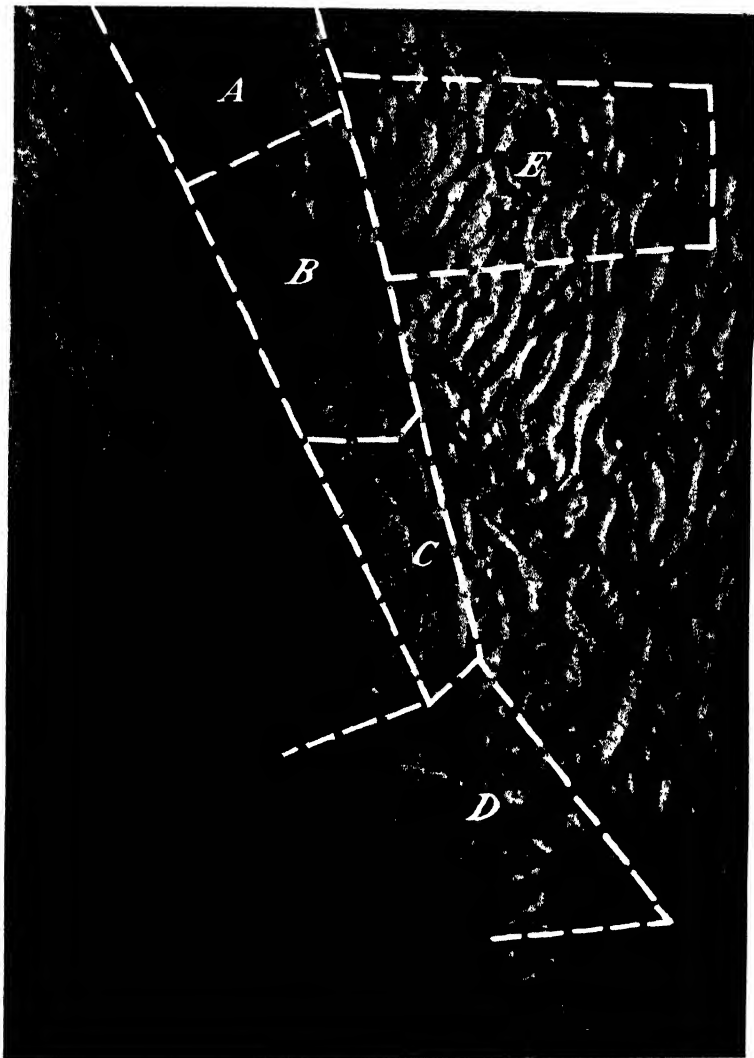


FIGURE 6.—Relief map of California and Nevada with quadrangles showing longest feasible units for forecasting stream flow in Sierra Nevada and Great Basin ranges. *A*, northern Sierra quadrangle; *B*, central Sierra quadrangle; *C*, southern Sierra quadrangle; *D*, Southern California quadrangle; *E*, Humboldt quadrangle.

lumne, Stanislaus, Tuolumne, and Merced Basins on the western slope. Crest stations representative of the major portion of the quadrangle were established, with outposts along the eastern side, four of the latter being on the crest of the Carson Range, which forms the eastern wall of the Tahoe Basin. On the western side, outpost stations

were established only in the Yuba Basin in the northwestern part of the quadrangle.

The southern Sierra quadrangle included all crest basins from the San Joaquin Basin to Tehachapi Pass, thus comprising the San Joaquin, Kings, Kaweah, and Kern Basins on the west and the long Owens Basin on the east. Special attention should be given to the surveys in the complex Kern Basin, which includes the long trough between the double crest of the high Sierra and the broad valley adjoining the Mojave Desert. The northern Sierra quadrangle comprised the upper Klamath, McCloud, Pit, and upper Sacramento Basins on the west and the minor Goose Lake Basin on the east. The mountains are relatively low and without commanding crests, and outpost stations are difficult to obtain. Moreover, the maximum flow of the streams occurs in February to May, instead of March to June and April to July as do the streams successively toward the south, but the curve of flow is greatly flattened by the retarding effect of the lava field. However, the snow fields of Mount Shasta and Lassen peak, aided by seasonal catch gages on the floor of the basin, might prove to be a factor of considerable importance in helping to forecast the flow.

FOOTHILL STREAMS AND SOUTHERN CALIFORNIA

The Cosumnes and Tule, the only foothill streams of major importance in the Sierra, resemble the northern Sierra streams in their early run-off. However, their curve of flow is sharper and the necessity of early surveys and short forecasts is consequently greater. The western outpost station of the Mokelumne at Bear River Reservoir would serve as a crest station for the Cosumnes; and Hackett Meadows, selected as one of the western outposts for the Kaweah-Tule, would serve reasonably well as a crest station for the Tule. The only possible outpost stations for either would be precipitation stations equipped with catch gages.

In southern California, where the ranges are comparatively low and the precipitation is light, many of the crest stations must depend upon catch gages for determining the total winter precipitation, and except in the case of underground flow, the forecast must often present the effect of individual storms, rather than the cumulative effect of the entire season. However, the presence of some high peaks, as in the San Gabriel Mountains, and the major importance of the sub-surface flow make the establishment of a fourth quadrangle, known as the Southern California quadrangle, desirable.

In this quadrangle the seasonal water must be classified under two heads: (1) The amount available from snow fields and the precipitation that falls upon them, and (2) the amount available from rain or quickly melting snow. Since the pruning loss from the first is far less than from the second, it follows that the net gain from the former is far greater and should be given greater weight in estimating water resources.

The net gain from the latter will naturally depend upon the heaviness of the precipitation and the previous dryness of the soil. The foregoing general plan is being carried out by the present California cooperative snow survey except that the local basin rather than the quadrangle constitutes the main unit.

INAUGURATING A SNOW SURVEY

LOCATING THE SNOW-SURVEY COURSES

In inaugurating the snow survey of a basin or drainage area, the initial steps should be to determine the chief tributaries of the draining stream and to locate snow-survey courses at points of major flow. In this way a large basin can be served with a minimum of effort. For example, the Humboldt Basin in Nevada (fig. 6) has a length of 350 miles and a drainage area of 14,200 square miles above Humboldt Lake, which receives its residual waters. But its run-off can be determined fairly well from four stations strategically placed in the Ruby and Charleston-Independence Mountains, these being parallel ranges 80 miles apart which furnish approximately 90 percent of the water of this basin.

The supplies from these two ranges bear approximately the ratio of 3.2 in favor of the higher and more rugged Ruby Range. Of the



FIGURE 7.—Ideal site for a snow course, Big Meadows, eastern outpost snow course of the Truckee River basin. (Meadow is in middle distance.)

feeders from the Charleston-Independence Ranges, Mary's River and North Fork furnish 84 percent of the total run-off on that side, and of the three from the Ruby Mountains, Lamoille Creek and South Fork furnish 86 percent from their group. Furthermore, South Fork furnishes twice as much water as Lamoille Creek. On the basis of these observations, there should be 4 groups of snow courses: 2 in the Ruby Range, 1 each along South Fork and Lamoille Creek, and 2 in the Charleston-Independence Ranges, along the headwaters of Mary's River and North Fork. The courses planned for the larger streams in each group will serve probably for the smaller streams as well. An ideal course site is shown in figure 7.

An example of another type is the Bow River Basin of Alberta, Canada, where the Canadian Meteorological Service inaugurated snow surveys in 1916-17. This basin is so alpine at its head in the Glacier National Park that a survey by the method of areas is physically impossible, and even a survey by the method of seasonal percentage is far from easy because of the difficulty of finding sites for stable courses. But an adjacent smaller basin of gentler contours has a

snow cover closely representative of that in the larger basin. Consequently, the seasonal percentages in the smaller basin can be safely applied to the stream flow of the Bow River Basin also.

In basins reaching far down from the crest of the range, crest and outpost stations should be established, the latter at a sufficient distance from the former to give a fair average of seasonal percentage at the two extremes of the basins.

ZONING

Recent years of light precipitation and premature melting have driven forecasters inevitably to zoning. The outposts which seemed to provide an index of oscillation horizontally furnished no clue vertically even when early run-off was plainly manifest.

The system of zoning or weighting depends upon the relative area in each watershed between fixed elevations. The lower limit of elevation represents the altitude of the stream-gaging station or lake level, and the upper represents that of the crest of the watershed. Three horizontal zones usually suffice.

These zones can readily be laid out on a topographic sheet, available for most watersheds, and their area determined by planimeter.

Representative snow-survey courses are established in each zone, and the seasonal percentage of snow cover for each zone is determined in terms of its own normal. The average of these zonal percentages when weighted for the relative area of its own zone represents the weighted average for the basin.

The drawback to this system is the present lack of snow-survey courses at lower levels, and the difficulty of finding a course at low levels that may not occasionally be bare at the time of surveys; for some index of snow cover must be had, however poor the season. Otherwise all definiteness is gone. Advantage is gained from using the system only when the zonal percentages differ from each other.

BRIDGING

In cases where funds are insufficient for surveying all the basins in a series, the method of bridging can be used with a fair degree of accuracy. This is merely using the average of the seasonal percentage of flanking basins as representing the seasonal percentage of the basin intersituated. Thus in the Walker Basin, in which crest snow-survey courses are remote from habitations, preliminary estimates of snow cover are made March 1 by the simple expedient of using the average of the percentages at the crest stations in the Mono and Carson Basins, though distant 60 miles apart, and averaging this with the seasonal percentage of the eastern outpost of the Walker Basin, which is readily accessible. Thus the chance of oscillation from any direction is avoided.

Occasionally, a final forecast has been made for the Carson Basin by simply averaging the forecasts for the Walker and Tahoe Basins, which flank it.

DEVELOPING A NORMAL

In the absence of a normal for either snow cover or run-off, a simple process is to obtain data for the current snow cover and stream flow and apply them provisionally as a standard to the data obtained

during the second and immediately succeeding years, until such time as a normal can be computed. Care should be taken to determine whether the run-off of this first or standard year has been abnormally affected by lack of normal precipitation or by other variable factors after melting began, for otherwise the resulting error would be perpetuated.

If rainfall measurements in the basin are not available, use should be made of those nearest or most reliable, for lack of rain sufficient to cause extreme variation in the run-off will be a general rather than a local phenomenon. If a satisfactory normal for stream flow is already at hand, so that the seasonal percentage of the first year is definitely known, this percentage can be considered as that of the snow cover also, provided no abnormal shrinkage has occurred in the former. From this percentage, the normal of the snow cover can be obtained at once.

A capital example of such pioneering is furnished by the experience of 1919-20 in the Humboldt Basin. The courses were 1 year old and some melting had occurred before the original measurements could be made. Furthermore, the succeeding stream flow, measured by the United States Geological Survey, had not been announced. On the assumption that the melting was 3 inches of water (based on observations in the Sierra Nevada), the original measurements were increased by this amount and were then used as a standard of comparison. On this basis, the snow cover of the season of 1919-20 was found to be 76 percent of that of the preceding season. On the other hand, there had been a probable shrinkage of 25 percent in run-off the preceding season due to general lack of precipitation, which might not occur in the present season. Consequently, a forecast of stream flow approximately equal to that of the preceding season was made, but owing to weather conditions this was reduced in the May forecast to 91 percent.

Although no definite statement of the acre-feet available could be made, the comparison with conditions in the previous season was even more comprehensible to the irrigationist, who still had a vivid recollection of its dryness. Later, the stream flow for 1918-19 (April to July) was announced as 115,690 acre-feet, or 49.6 percent of normal. The estimate for 1919-20 was immediately changed to 45.1 percent or 105,278 acre-feet. The actual run-off was 105,950 acre-feet, or 45.4 percent. However, this apparent closeness may be merely deception, for the gaging station is below diversions.

A provisional normal may be based on as few as three seasons' measurements, provided the seasons are complementary. Thus, in the Tahoe Basin the seasons 1909-10 to 1911-12 and 1912-13 to 1914-15 consisted of a heavy, an average, and a light snowfall each, and their normals for crest and eastern outpost stations, measured in inches of water, bear a close resemblance to each other and to the 6-year normal. (The figures, in inches, for the crest were 45.09 and 42.99 for the respective 3-year periods 1909-10 to 1912 and 1912-13 to 1914-15, and 44.04 for the 6-year period 1909-10 to 1914-15; those for the eastern outpost for the same periods were, respectively, 26.14, 27.02, and 26.56.) This rare occurrence has not been repeated and only recently has the average of the snow covers since 1915 approached normal.

Years of very excessive snow cover or run-off should be omitted in forming a normal unless the normal is based upon records for a very large number of years. Otherwise the "reasonable expectation" will be set too high and chronically subnormal years will result. For this reason, in all computations made in the Sierra Nevada surveys, seasons above 200 percent have been omitted temporarily. Such high records include the rise of 225 percent in Lake Tahoe in 1906-07 and the tremendous flows in the Kern River of 345.4, 302.7, and 390.3 percent in 1905-06, 1908-09, and 1915-16, respectively.

REVISED PARALLEL NORMALS

Where possible, the normal for snow cover and its run-off should be based on the same period of years, and that period should be reasonably long. A normal of 24 years has been computed for the run-off of the Truckee Basin, and the various normals for snow cover there have been expanded to correspond. This normal could have been lengthened still further by including the dry years since 1928, but it was feared that the normal for run-off would thereby be depressed too much. However, comparison with a 41-year record in the Yuba Basin and physical evidence of low lake levels at an earlier period in Eagle Lake not far north indicate that such would probably not be the case.⁶

This 24-year normal of run-off in the Truckee Basin was further used as a standard for expanding the normals for both run-off and snow cover in neighboring basins.

A 24-year normal was likewise computed for run-off in the Humboldt Basin, but owing to the effects of diversion, and also probably to the spotted character of desert precipitation, it did not make a satisfactory standard for expanding the normals in its tributaries.

SPRING AND SUMMER PRECIPITATION STATIONS

In each basin, precipitation gages sheltered from the wind and of sufficient capacity to catch either snow or rain should be centrally located to determine the average precipitation over its area during the period of melting and late summer and autumn run-off. A gage at a central settlement will usually suffice, but occasionally a seasonal gage set farther into the basin will be more satisfactory.

FORECASTS

Three forecasts of run-off each season will meet all ordinary needs, except when reservoirs are used for the dual purpose of flood catchment and irrigation storage. Then surveys and forecasts should begin with the first heavy snowfall and continue at intervals throughout the season. The first forecast is made early in April and represents merely the seasonal percentage of the snow cover at its maximum stage before melting has set in. The second is made early in May, when the probable effect of weather on the run-off can be anticipated and the estimate of probable run-off corrected for abnormal shrinkage. The third is made early in June, when the run-off of April and May furnishes a clue to the balance available.

⁶ HARDING, S. T. CHANGES IN LAKE LEVELS IN THE GREAT BASIN AREA. . . Civil Eng. 87-90, illus. 1935.

Such forecasts will be requisite to the necessary control of the Truckee River under the new plan of additional storage and careful regulation being formed to serve divergent interests below.

The snow surveys made early in May and in June indicate the relative amount of snow still unmelted, and when a normal for melting has ultimately been obtained these surveys will furnish a check on the forecasts. These later surveys need be made at only a few central stations, but the courses should preferably be on level ground to indicate average conditions of melting at their altitude.

Temperature has such an immediate effect upon the run-off that it is always a disturbing factor in attempts to forecast the rate of run-off for any considerable period. In the case of reservoirs and lakes whose normal decrease from maximum stage due to evaporation is known, forecasts of available water for irrigation and power can be made with fair certainty until autumn, when the new season's storms begin.

ACCURACY OF SNOW SURVEYS AND FORECASTS OF STREAM FLOW

The following summary of the accuracy of 63 snow surveys and forecasts corrected May 15 for probable departure in precipitation during run-off indicates that two-thirds were within 10 percent of accuracy and almost four-fifths within 15 percent. This result was attained with old normals. Of the 6 basins included in the summary, one was a lake basin, another was affected by large diversions above the point of gaging, a third lacked outpost stations. Zoning was employed only in the more recent years of the series. The accuracy of the forecasts—that is, the variation between forecasts and actual run-off in percentage of normal run-off—is shown for various percentage classes in the following tabulation:

Accuracy within following percentage based on normal run-off:	Number of forecasts
0 to 5 percent.....	27
5 to 10 percent.....	14
10 to 15 percent.....	7
15 to 20 percent.....	6
20 to 25 percent.....	5
25 to 30 percent.....	3
30 to 35 percent.....	1
Total.....	63

ORGANIZED EFFORT ESSENTIAL

Single-basin surveying is sometimes unavoidable, but larger public service with increased accuracy and relatively decreased expense can be rendered by wide-area surveying with a central agency directing the work. In this way overlapping can be avoided. Furthermore, early knowledge of the latent water resources of the range would permit power companies in deficient basins to make adjustments by "tying-in" with their more fortunate associates.

Where watersheds cover two or more States, cooperative snow surveying is essential either by the Federal Government or by the States. Such watersheds are the Sierra Nevada series and the Colorado and Columbia Basins. Water districts, power companies, and municipalities should be included in the cooperation to obtain understanding and cooperation from the public.

COST OF SNOW SURVEYS

The cost of surveying a basin depends upon the number of its course and their accessibility. In the Tahoe Basin, the "control" basin of the central Sierra section, with its seven stations and a circuit from Reno of approximately 320 miles by train, automobile, ski, sledge, and motorboat, the April survey requires the equivalent of 13 days by a party of two persons, or proportionately less by the three parties that usually do the work. In the Yuba and Carson Basins, 4 to 6 days are required. But in the Walker Basin 10 days of strenuous effort and 110 miles of snowshoeing are necessary to cover the two forks and reach the almost inaccessible crest stations between the Walker and Tuolumne Rivers. Surveys on May 1, because of their small number,



FIGURE 8.—Refuge hut on slope of Mount Rose at an altitude of 9,000 feet, headquarters of the snow surveys on Mount Rose.

require far less time, being confined mainly to the crest stations in the Carson, Tahoe, and Yuba Basins.

The present problem is to penetrate the higher basins of the Sierra Nevada far enough to make an accurate determination of their snow cover. To accomplish this chains of refuge huts a day's march apart must be constructed and provisioned. The only requirement is a preliminary winter reconnaissance to find sites where the snow lies sufficiently shallow to permit entering the huts without effort. To make the latter problem simpler, a "Santa Claus chimney" may be constructed as an entrance. The refuge-hut system is now well established (fig. 8).

To the expense of the actual surveys must be added the initial cost of snow samplers and snow traveling equipment, this cost depending on the number of parties in the field. The sum of \$15,000 was suggested as the annual expense for conducting the entire system of surveys

throughout the Sierra Nevada. By means of contributions from co-operators, the legislative appropriations are being kept within this limit.

LIMITATIONS

The snow surveys are limited in accuracy corresponding to the increase in the relative precipitation during run-off. Since this relative precipitation is least on the Pacific coast and increases steadily to the Continental Divide, the parallelism of the snow cover and run-off likewise diminishes toward the east. Thus the ratio of precipitation during run-off (April-July) to the snow cover or precipitation during November-March varies from 18.2 percent in the Sierra Nevada to 85.7 percent in the Continental Divide and 238.2 percent in the Great Plains at Denver. This disparity with coast conditions persists in Montana⁷ and is even greater in Canada, where the percentage reaches 284.9 percent at Calgary.

However, since the snow cover during melting has the character of continuous precipitation, its effectiveness far exceeds that of a similar quantity of occasional rains, which are partly wasted in repriming the

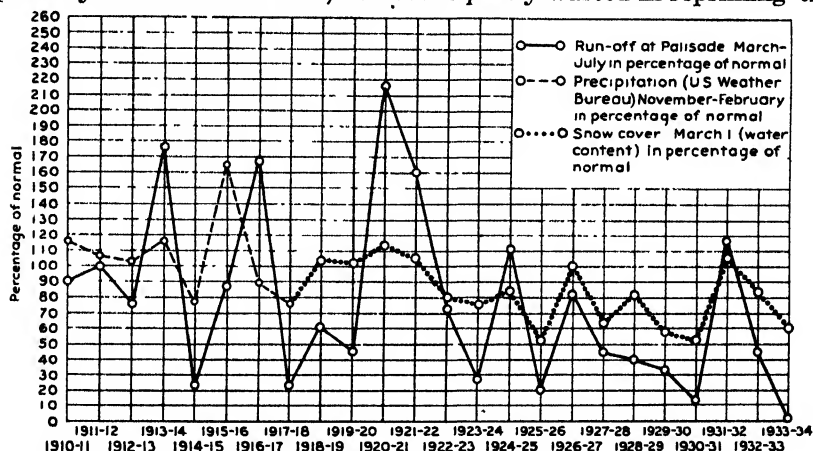


FIGURE 9.—Comparison of snow cover and run-off, Truckee River Basin, exclusive of Lake Tahoe, 1912-13 to 1933-34

soil. Thus the percentage of loss in run-off due to complete lack of normal summer precipitation will probably range from 16 percent in the Sierra Nevada to approximately 30 percent on the Continental Divide. In the Plains region, the loss may vary from 75 to 90 percent.

Figures 9 and 10 show the degree of steadiness between the snow cover and the run-off under ideal conditions of heavy snow cover, shallow stream bed, and relatively light summer precipitation, and the difficult conditions of light snow cover, alluvial stream channel, and relatively heavy summer precipitation. But even in the latter case, the analysis of the affecting factors has brought a fair degree of accuracy in forecasting.

SNOW SURVEYING IN PRACTICE

The simple principles already outlined form only a part of the problem of snow surveying, for the requirements are frequently complex.

⁷ The ratio of November through March precipitation to that of April through August is as follows: Billings, 1.2.76; Havre, 1.3; Helena, 1.2; Miles City, 1.2.7.

Therefore, adjustments and experimentation are essential in each phase of the work. A simple enumeration of the problems already encountered⁸ opens a wide field, even though uses other than for forecasting stream flow are omitted. Of these problems one was a forecast of how often Lake Tahoe would fall below its outlet during the next 50 years under present regulation. Another was an estimate as to how long it would require Lake Tahoe to regain its normal level if artificially drawn down. A third was an estimate as to the probable normal run-off of the East Walker River, Nev., for which only 2 years of record existed. A fourth was the probable run-off high up on the West Walker based upon the run-off much farther down the stream. A fifth was the relation of run-off to precipitation on the Continental Divide.⁹

Special snow-survey systems have also been worked out for the Klamath and Tule Basins in Oregon and California, for the Coeur d'Alene in Idaho-Washington, and for the Bow in Canada. The first basin was typical of streams flowing from a dry to a wet zone.

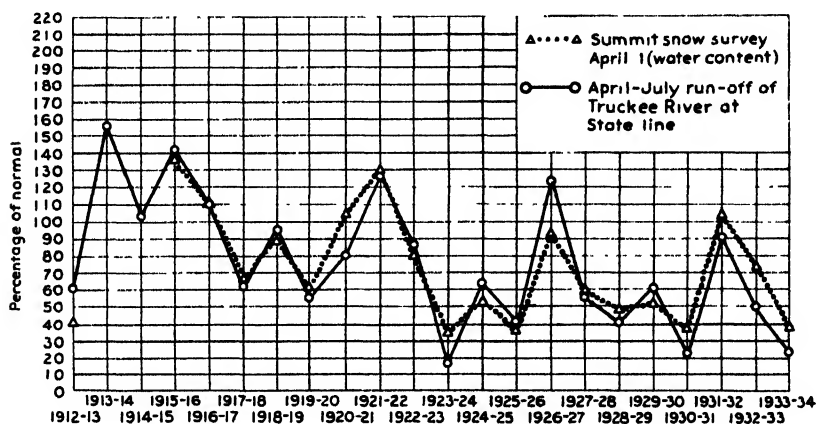


FIGURE 10.—Comparison of snow cover and run-off, upper Humboldt Basin, 1910-11 to 1933-34.

The second represented a dry lake bed that could easily be flooded. The third was a basin whose storage was small as compared with its total discharge, and consequently required careful forecasting to conserve late waters without overflowing the reservoir. The fourth represented run-off from snow and summer rains in about equally effective quantities. Its source was so rugged that parallel and less rugged watersheds had to be sought for the snow surveys.

At the present time, a cooperative effort is being made to improve forecasting in the Humboldt Basin, Nev.,¹⁰ where scant snow cover, relatively heavy precipitation during run-off, numerous diversions above gaging stations, alluvial stream bed, tortuous channel, and tight dams create obstacles for the forecaster. The universality of the problem makes this basin an ideal laboratory for the study of snow surveying.

⁸ Unpublished.

⁹ CHURCH, J. E., and JONES, E. H. PRECIPITATION AND RUN-OFF AT THE CONTINENTAL DIVIDE. *Engin. News-Rec.* 94:190-95. (Discussion by H. P. Boardman, p. 195.)

¹⁰ CHURCH, J. E. SNOW SURVEY PROBLEMS IN THE HUMBOLDT BASIN, NEVADA. Reprinted from State Engineer's Report, 1932, Distribution in the Humboldt Basin.

FORECAST SYSTEMS FOR THE COLORADO AND COLUMBIA RIVER BASINS

The following analysis of the Colorado and Columbia River Basins has been made as a basis for the snow-survey systems that have now become essential for their regulation.

COLORADO RIVER BASIN

The Columbia and the Colorado together drain the entire western slope of the Rocky Mountains from New Mexico into Canada (a distance of approximately 1,300 miles) and furnish an annual water flow of approximately 169,000,000 acre-feet at the ratio of 9:1. The Colorado has a run-off of 17,500,000 acre-feet—a large run-off, but relatively small when compared with that of the Columbia.

Of the two projects, the forecasting of the Colorado is more pressing because of the approaching completion of the Boulder Canyon Dam. This project is also far simpler, for unlike the Columbia, the Colorado, with the negligible exception of the Gila, rises entirely in the highlands of the Continental Divide and receives practically no additions toward its mouth. Thus the area of 225,000 square miles above Yuma, Ariz., is reduced for forecast purposes by about one-half, and the crest line of 760 miles is reduced to 550 miles. Furthermore, 3 tributaries—the Green, the Grand, or upper Colorado, and the San Juan—furnish 87.1 percent of the mean annual run-off at Yuma of 17,500,000 acre-feet; that is, about 15,000,000 acre-feet. Of the 15,000,000 acre-feet thus furnished, 2 of these 3 tributaries or feeders, the Green and the Grand, or upper Colorado, furnish 12,500,000 acre-feet, or practically 82 percent. In other words, the Green and the Grand or upper Colorado, furnish 71 percent of the entire flow at Yuma. The mean run-off of these streams as computed in 1921 is as follows:

	<i>Acre-feet</i>
Green River.....	5, 800, 000
Grand, or upper Colorado.....	6, 600, 000
San Juan.....	2, 700, 000

Since the San Juan is highly erratic in its run-off and very important in the early season operations of the Boulder Canyon reservoir, all three tributaries should be included in any detailed plan of snow surveying. There will thus be included the watershed crest from the Colorado-New Mexico line almost to Yellowstone Park and then southwesterly to central Utah. Hence, of the seven States immediately interested in the Colorado River—Wyoming, Colorado, Utah, New Mexico, Arizona, Nevada, and California—only Wyoming, Colorado, and Utah contain the primary and essential snow-survey courses. Simpler still, the joint forecast for these three tributaries will become the forecast for the main Colorado below. Precipitation in the semiarid region below the tributaries can have no more than a minor effect.

Unlike the Columbia, the Colorado has far more irrigable land tributary to it than it can serve. Consequently, close regulation of this stream will be essential in order to guard against floods and yet give maximum service to irrigation and power. To accomplish this, a snow-survey system should be begun immediately and developed to highest efficiency as the maximum use of the stream draws near.

COLUMBIA RIVER BASIN

The mean annual run-off of the Columbia at The Dalles is approximately 151,500,000 acre-feet. This tremendous flow of the Columbia is furnished by three principal tributaries—the upper Columbia with the Kootenay (50,000,000 acre-feet), the Clark Fork-Pend Oreille (19,000,000 acre-feet), and the Snake (45,000,000 acre-feet). The combined system covers with a more or less complete network the entire arid region of Idaho, Oregon, and Washington, and thus guarantees to these States a permanent foundation for agricultural and power development. The chief problem, especially downstream, will be the lifting of water to the highlands, and its solution may be the power ability of the stream itself.

The three tributaries mentioned (the upper Columbia, Kootenay; the Clark Fork-Pend Oreille; and the Snake) supply 77 percent, or about 117,000,000 acre-feet, of the total annual flow of the Columbia at The Dalles, and their individual basins are so large and their flow so abundant that at least two of them have become centers for a series of great reclamation projects. Of the tributaries, the Spokane has long been the source of interstate power.

The problem of forecasting the April to July run-off of the Columbia is virtually the problem of forecasting the run-off of its individual feeders, for the interests served are on the tributaries rather than on the main stream. However, the collective forecast for the feeders would represent the forecast for the main stream. This is shown by the record of 1913 to 1921. During that period the maximum annual variation between the collective run-off of the major feeders and the run-off of the main stream at The Dalles was within 7 percent; and the maximum variation for April to July was within 11 percent, although divergences of 20 to 35 percent frequently occur between the tributaries themselves. On the basis of fragmentary records, a similar closeness of agreement prevailed throughout the preceding decade. However, forecasting for even the individual feeders is far more complex than on the Colorado. Precipitation during April to July grows relatively heavier with increase in distance from the Pacific coast, and the snow cover on the upper Columbia watershed melts slowly during this period, thus catching and ultimately transmitting to the stream the bulk of the precipitation. Therefore, the snow cover on April 1 represents the minimum rather than the probable flow of the stream.

The lower Columbia drains the Cascade and Coast Ranges, which are here of low elevation and transmit the bulk of their snow immediately to the streams. For instance, 57 percent of the run-off of the Willamette occurs in December to March and 27 percent in April to July. Furthermore, the precipitation on the Willamette watershed is relatively light during April to July and adds little to the summer flow in the lower Columbia. On the other hand, the flow of the Columbia above The Dalles is only 17 percent in December to March and 61 percent in April to July. Consequently, the upper and lower Columbia are complementary to each other. Whatever late spring and summer rise occurs in the Columbia will be due to the snow on the Continental Divide. On the other hand, except for the influence of the chinook, the high water in winter should be due to heavy precipitation in the Cascade and Coast Ranges and should occur mainly in the lower Columbia and its immediate tributaries.

SUMMARY

Snow, while falling, is the plaything of wind currents and so resists exact measurement as precipitation. Consequently, it cannot satisfactorily be measured as is rain in a catch gage. On the other hand, after falling, snow is an elastic substance that cannot be measured in terms of depth only as is rain. To meet these two problems a snow sampler was devised that would quickly measure not only the depth of the snow but its water content as well.

However, in the semiarid West streams are fed almost entirely by snow fields, and the water must be carefully apportioned. Even if it were possible to determine the exact quantity of water in the snow field or the net amount that would quickly measure not only the stream draining it, it would not be feasible to do so.

Recourse was therefore had to the percentage system of forecasting, for it was found that the relative snow cover throughout a basin was the same wherever measured provided neither drifting nor melting had occurred. To avoid the errors that might arise from drifting, long courses with 25 to 50 samplings were employed, and the average was taken as indicating the snow at that point. Melting was rare and was met by a system of altitude zones. It was now merely necessary to maintain a single fixed course in each zone to determine the relative amount of snow in the basin. Frequently, if melting had not occurred, a single course would serve all zones. This course, if averaged for a fair number of years, would give a "normal" or "mean" water content by which to estimate the percentage relationship of coming seasons. This percentage was obtained by dividing the new snow cover by the mean snow cover; that is, by the average of all the snow covers preceding.

This percentage relationship was found to correspond closely to a similar percentage relationship of run-off in the stream below into which the snow field was discharging. Thus a 75-percent snow cover on April 1 assured an approximately 75-percent run-off during the months of April through July, after which the streams rapidly run dry. The only divergence found was caused by lack of the precipitation that normally falls during the run-off season. This, however, can be fairly well estimated after the first 6 weeks, and corresponding corrections made in the estimate.

So uniform is the snow cover for great distances along the axis of the Sierra Nevada, that usually variations of less than 20 percent occur over distances of 100 miles. Thus the run-off forecast for one stream can be applied also to its neighbor and even to its neighbor's neighbor, and likewise to streams on opposite sides of the crest.

Accuracy within 10 percent in the forecast is usually possible. Even in the case of a single group of snow courses at Summit Station at the crest of the Truckee Basin in the Sierra Nevada, the divergence between snow cover and run-off in 12 years out of 17 was less than 10 percent.

A larger comparison under difficult conditions indicates similar accuracy. In 63 forecasts for the Truckee, Tahoe, Carson, West Walker, south Yuba, and Mokelumne Basins, covering 19 years, 41, or practically two-thirds, were within 10 percent of accuracy, and all were within 31 percent. Twenty-seven, or nearly one-half, were within 5 percent. This system is known as the percentage, or Nevada, system.

Snow surveys are limited in accuracy in proportion to the increase in the relative precipitation during run-off. Since this relative precipitation is smallest on the Pacific coast and increases steadily to the Continental Divide, the parallelism of the snow cover and run-off likewise diminishes toward the east. Thus the ratio of precipitation during run-off (April through July) to the snow cover or precipitation during November through March varies from 18.2 percent in the Sierra Nevada to 85.7 percent in the Continental Divide and 238.2 percent in the Great Plains at Denver. This disparity with coast conditions persists in Montana and is even greater in Canada, where the percentage reaches 284.9 percent at Calgary.

However, since the snow cover during melting has the character of continuous precipitation, its effectiveness far exceeds that of a similar quantity of occasional rains, which are partially wasted in repriming the soil. Thus the loss in run-off due to complete lack of normal summer precipitation will probably range from 16 percent of normal run-off in the Sierra Nevada to approximately 30 percent on the Continental Divide. In the Plains region, the loss may vary from 75 to 90 percent.

Notwithstanding its limitations, snow surveying is valuable in forecasting the minimum run-off from a watershed. Such is its purpose where the snow cover is regarded merely as a supplement to ground-water storage and summer rains.

NEMIC PARASITES AND ASSOCIATES OF THE MOUNTAIN PINE BEETLE (*DENDROCTONUS MONTICOLAE*) IN UTAH¹

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INTRODUCTION

The mountain pine beetle (*Dendroctonus monticolae* Hopk.) has been known for many years in the lodgepole pine belts of Utah. In 1929 outbreaks of this pest, affecting many thousands of trees, began in widely separated localities.

The most serious outbreaks were on Blacks Fork, Horse Creek, and Smiths Fork, near the Utah-Wyoming line in Summit County, Utah, and Uinta County, Wyo. Another severe infestation developed in the upper Provo River Basin east of Kamas, Utah, between Shingle Creek and Broadhead Meadows. These outbreaks spread rapidly from the original centers of infestation during the years 1930 to 1932. At the end of this time they were checked by a control program inaugurated by the Forest Service, United States Department of Agriculture, which consisted of surveying the areas and burning the infested trees. In the course of this work the nemas described in this paper were collected.

HISTORICAL REVIEW

A small outbreak of the mountain pine beetle on lodgepole pine (*Pinus contorta* Loudon) occurred on Henrys Fork, Summit County, Utah, in 1924-25. This outbreak apparently was diminishing when control measures were applied and the beetles were practically eradicated. A similar outbreak on Rock Creek, Duchesne County, Utah, about 1915 to 1917 was reduced by unknown natural agencies.

Evidences of previous outbreaks are present in certain localities. "Cat-faced" (scarred) trees with pitch-preserved beetle borings are numerous in the Henrys Fork territory, and the annual rings show that the outbreak occurred between 1870 and 1880. In the Horse Creek territory there is similar evidence of an outbreak about 150 years ago.³ The rarity of such outbreaks in this locality indicates that climatic conditions, diseases, or parasites have generally effected sufficient control to hold the beetles in an endemic stage and that, unlike conditions which exist in lodgepole pine forests in Idaho and Montana, only occasionally are conditions favorable for serious outbreaks. The studies herein presented show that nematode parasitism was negligible during the outbreak under discussion.

Published information on the nematode parasites and associates of the mountain pine beetle is limited to a recent paper by Steiner⁴ in

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² The writer is indebted to A. G. Nord, supervisor, and Blaine Betenson, assistant supervisor, of the Wasatch National Forest, Utah, who suggested this problem and secured much of the material through the forest rangers under their direction.

³ NORD, A. G. Oral communication.

⁴ STEINER, G. SOME NEMIC PARASITES AND ASSOCIATES OF THE MOUNTAIN PINE BEETLE (*DENDROCTONUS MONTICOLAE*). Jour. Agr. Research 45: 437-444, illus. 1932.

which three associate species, *Diplogaster occidentalis* Steiner, *Aphelenchoides conurus* Steiner, and *A. acroposthion* Steiner were described from Idaho.

NEMAS COLLECTED

Collections of larvae and adult beetles, together with bark from infested trees, were secured from four localities in Utah, namely, Blacks Fork, Horse Creek, Smiths Fork, and the upper Provo River Basin. Ten species of nemas, 9 of them new to science, were secured from this material. The distribution of these species is given in table 1.

TABLE 1.—Distribution of 10 species of nemas, including 9 new species, found in 4 localities in Utah

Species of nema	Distribution ¹ of species in locality indicated			
	Blacks Fork	Horse Creek	Smiths Fork	Upper Provo River Basin
<i>Aphelenchulus reversus</i> , n. sp.	+	+	+	+
<i>Anguillulina pinophila</i> , n. sp.	+	+	+	+
<i>Anguillulina magnicauda</i> , n. sp.	+	+	—	—
<i>Aphelenchoides brachycephalus</i> , n. sp.	+	+	—	—
<i>Aphelenchoides talonius</i> , n. sp.	+	+	+	+
<i>Aphelenchoides tenuidens</i> , n. sp.	+	+	+	+
<i>Aphelenchoides latus</i> , n. sp.	+	+	+	+
<i>Panagrodontus dentatus</i> , n. g., n. sp.	+	+	+	+
<i>Diplogaster pinicola</i> , n. sp.	+	+	—	+
<i>Rhabditis obtusa</i> Fuchs, 1915.	—	—	—	+

¹ Plus (+) sign indicates presence; minus (—) sign, absence.

Samples of bark from 20 uninfested trees adjacent to those infested with beetles were also examined. The fact that not one species of the nemas associated with the beetles was found in these samples indicated that the nemas might be dependent upon the beetles for transportation from tree to tree and for providing suitable living quarters.

NEMIC ENDOPARASITE OF THE MOUNTAIN PINE BEETLE

A single endoparasitic species belonging to the genus *Aphelenchulus* was found in every locality investigated. About 2 percent of both adults and grubs of the beetle were each infested with 1 to 11 female nemas and, in some instances, scores of eggs, larvae, and immature females. This genus previously has been represented by a single species, *A. mollis* Cobb, 1920,⁵ a parasite of the wood-boring beetle, *Cyllene picta* Drury.

APHELENCHULUS REVERSUS, N. SP.

Eggs.—Deposited before segmentation. Size variable, 30 μ by 60 μ to 42 μ by 90 μ . Several hundred deposited by each female in the body cavity of the grub or adult beetle. Segmentation and hatching occur immediately after deposition.

Newly hatched larvae.—Length 0.22 to 0.30 mm; width 12 μ to 16 μ . Cuticle finely striated. Lip region rounded and expanded (fig. 1, A). Tail conoid to the small blunt terminus (fig. 1, B). Spear exceedingly slender, without basal knobs.

⁵ COBB, N. A. ONE HUNDRED NEW NEMAS. Contributions to a Science of Nematology, IX, pp. 301-302, illus. Baltimore. 1920.

Esophagus a slender tube, narrowing as it passes through the nerve ring, then gradually expanding and merging with intestine. Excretory pore a little posterior to nerve ring.

Second-stage larvae.—Measurements of recently molted specimens:

1.5	16.	20.	Juv.	95.	
1.2	3.4	3.7	5.	3.	0.35–0.42 mm

Similar in appearance to the young larvae except for the uniformly tapering anterior end (fig. 1, C) and the developing gonads. Genital primordium visible at beginning of first molt (fig. 1, B). From it the single ovary develops forward until it is about half as long as body, its terminus reflexed a distance equal to 3 to 5 body widths. A prominent gland usually is visible just back of the nerve ring. During this stage little or no increase in body length but marked development in width.

Intermediate forms between this stage and the adults were not found. Apparently it is during this portion of the life cycle that the nemas leave their

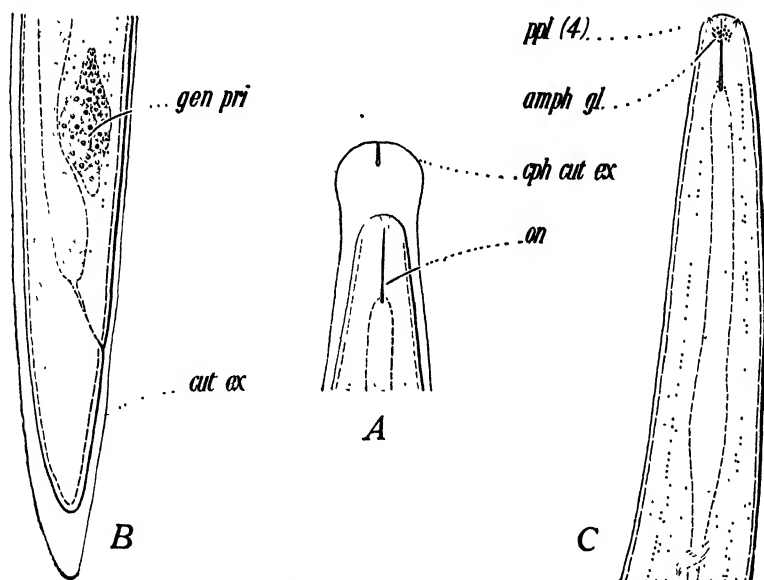


FIGURE 1.—*Aphelenchulus reversus*. A, Anterior end of molting first-stage larva: *cph cut ex*, Larval cephalic cuticle; *on*, spear. $\times 1,000$. B, Posterior end of molting first-stage larva: *gen pri*, Genital primordium; *cut ex*, larval caudal cuticle. $\times 1,000$. C, Anterior end of second-stage larva: *ppl (4)*, Labial papillae; *amph gl*, amphidial glands. $\times 1,000$.

hosts and transfer to other beetles or grubs. However, none was found outside the bodies of the hosts and the method of transfer remains unknown.

Females from grubs and adult beetles.—Length 1.0 to 1.8 mm; width 50μ to 180μ . Vulva 94 to 96 percent. Body bent dorsally, more or less cylindrical throughout greater part of its length but tapering conspicuously at the very narrow lip region, which is not set off in any manner. Cuticle annulated near the head and at the terminus; on some specimens annules conspicuous, on others almost invisible. Body constricted at vulva, especially ventrally. Tail broad, bearing dorsal, hornlike, annulated terminal projection which actually is the upturned original tail of the immature nema. This "horn" apparently becomes upturned as body distends with growth of the internal organs, and pressure is relieved on the ventral side when the broad vulvar opening is formed at the last molt. The four labial papillae almost invisible even from a face view. The amphids lie close to oral opening. Four large glands are prominent feature of head region. Spear 12μ to 14μ long, slender, with short ventrally located aperture. Knobs of the spear vary from obscure to distinct. Lumen of esophagus can be traced only a short distance from the spear. A series of 15 to 18 pairs of conspicuous lateral structures distributed throughout the body. Vulva a broad transverse slit.

Three glands lie opposite vulva, causing constriction of the organs. Anus and rectum absent. Ovary extending forward about three-fourths the length of body, then reflexed a distance equal to 1 to 2 body widths. Oviparous. Females generally burst when removed from the host (fig. 2).

Diagnosis.—Oviparous *Aphelenchulus* with dorsally bent body bearing a prominent, upright terminal "horn." A series of 15 to 18 pairs of conspicuous lateral structures distributed throughout the body. Parasitic in the body of *Dendroctonus monticolae*.

NEMIC ECTOPARASITES AND ASSOCIATES OF THE MOUNTAIN PINE BEETLE

Three nemic species, *Aphelenchoides latus*, *A. tenuidens*, and *Panagrodonus dentatus*, were found living under the elytra of the beetles. In all instances only the larvae were found in this position, the adults

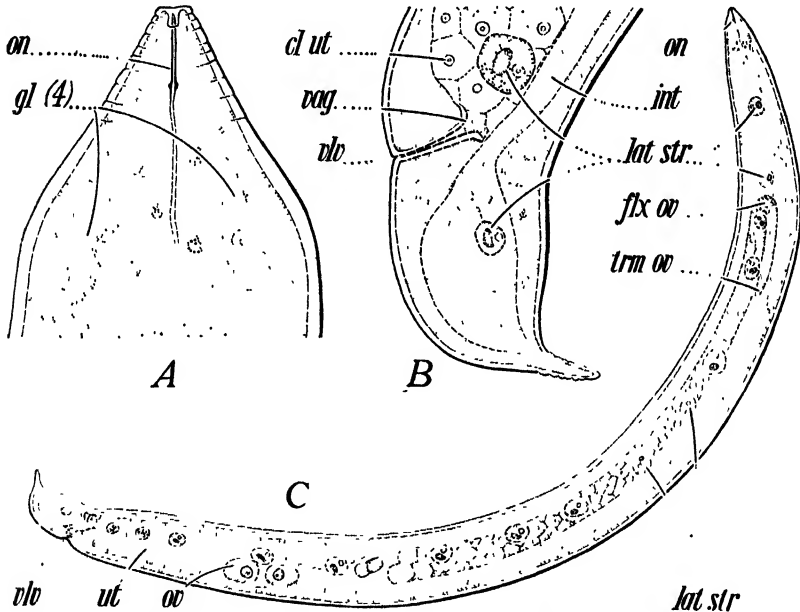


FIGURE 2.—*Aphelenchulus reversus* (female). A, Anterior end: on, Spear; gl (4), 2 of 4 glands near head $\times 800$. B, Posterior end: cl ut, Cell of uterus; vag, vagina; lat str, lateral structure; int, intestine; vib, vulva. $\times 400$. C, Female: on, Spear; lat str, lateral structure; flx ov, flexure of ovary; trm ov, terminus of ovary; ov, egg; ut, uterus; vib, vulva. $\times 100$.

living in the frass. It would seem that such ectoparasitism could cause but little injury or inconvenience to the host and that the principal purpose is to assure transportation to new locations when the beetles abandon dying trees.

Recent borings contained fewer nemic species than did the older ones, a fact which indicates that some species may be carried by secondary insects (Staphylinidae, Tenebrionidae, Ipidae, Histeridae, etc.) which invade the bark soon after the trees are attacked by the mountain pine beetle.

ANGUILLULINA PINOPHILA, N. SP.

Anguillulina pinophila (fig. 3) appeared in every collection made, usually in large numbers. It was the only species found in many trees, especially those in which the beetles had recently become established.

Typically plant parasitic in appearance, it apparently lives only in the tunnels, the lining cells of which are the most probable source of food. Because of the instability of the frass, a spear-bearing nema would experience difficulty in feeding upon it. None of these nemas was found in the bark or in soil about the bases of trees adjacent to those infested with beetles, indicating that the nemas are entirely dependent upon the beetles for transportation.

Anguillulina pinophila is closely related to *A. major* (Fuchs, 1915) new comb. (synonym, *Tylenchus major* Fuchs, 1915),⁶ from which it differs because of its narrower, more definitely set-off lip region and coarser striae. In some respects it resembles *A. dipsaci* (Kühn, 1858) Gerv. and V. Ben., 1859; *A. dendrophila* (Marcinowski, 1909) Goodey,

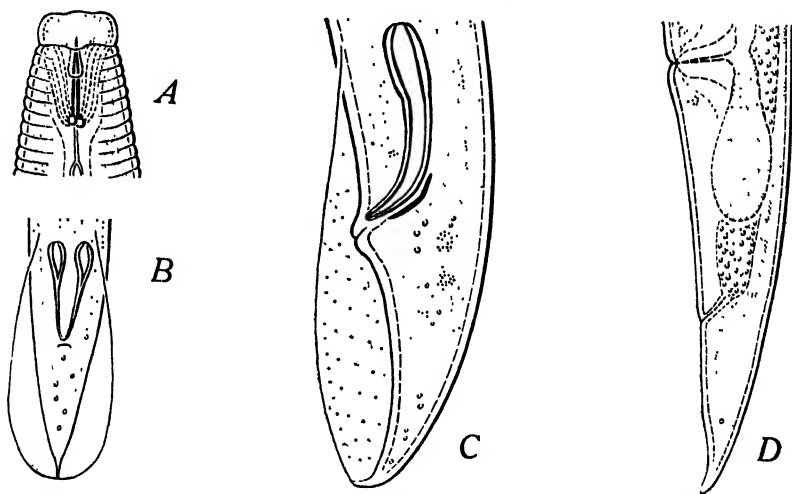


FIGURE 3.—*Anguillulina pinophila*. A, Head $\times 1,030$. B, Male tail, ventral view $\times 500$. C, Male tail, lateral view $\times 1,000$. D, Posterior portion of female $\times 500$.

1932; and *A. radicolica* (Greeff, 1872) Goodey, 1932. It differs from these species in the form of the lip region and male tail.

Measurements.—

0.5	5.	9.	W	⁸⁰ 91.3	97.	1.5–2.5 mm ?
0.4	1.6	2.1	4.	2.6	1.4	
1.	9.	16.	⁶⁵ M	97.2		
0.8	2.	2.5	3.	1.8		1–1.5 mm

Size rather variable, females usually considerably larger than males. Cuticle finely striated. Wing area smooth and refractive. Lip region rather flat, almost twice as wide as high, set off by a slight constriction. Spear a little longer than width of lip region, with small, though definite, basal swellings. Esophagus typical, median bulb one-half to two-thirds as wide as neck. Vulva a deep transverse slit. Ovary outstretched, variable in length, sometimes reaching median bulb of esophagus. Posterior uterine branch reaching one-half to three-fourths the distance to anus. Females approaching senility occasionally oviparous. Testis outstretched. Spicula three-fourths as long as tail, most arcuate in distal half. Gubernaculum thin, flat, arcuate, about one-fourth as long as spicula.

⁶ FUCHS, G. DIE NATURGESCHICHTE DER NEMATODEN UND EINIGER ANDERER PARASITEN. 1. DES IPS TYPOGRAPHUS L. 2. DES HYLOBIUS ABIETIS L. Zool. Jahrb., Abt. System., Geogr. u. Biol. Tiere 38: [109]–222, illus. 1915.

⁷ The letter W, occasionally used in formulas, denotes the widest portion of the body.

Female tail 2 to 3 times as long as anal body diameter, usually rather uniformly conoid to small rounded terminus. Phasmids a little posterior to middle of tail. Distance between vulva and anus variable. Male tail ventrally arcuate, uniformly conoid to pointed terminus. Bursa enveloping tail from terminus to a point opposite proximal ends of spicula.

Diagnosis.—*Anguillulina* of plant parasitic type from tunnels of mountain pine beetle. Lip region truncated, almost twice as wide as high, set off by a slight constriction. Spear slightly longer than width of lip region, with small basal swellings. Spicula three-fourths as long as tail. Bursa completely enveloping tail. Measurements as given above.

ANGUILLULINA MAGNICAUDA, N. SP.

A single female of *Anguillulina magnicauda* was found associated with *A. pinophila* in beetle frass from a tree in the Horse Creek district. The specimen is so outstanding in its characteristics that it appears justifiable to base a new species upon it (fig. 4).

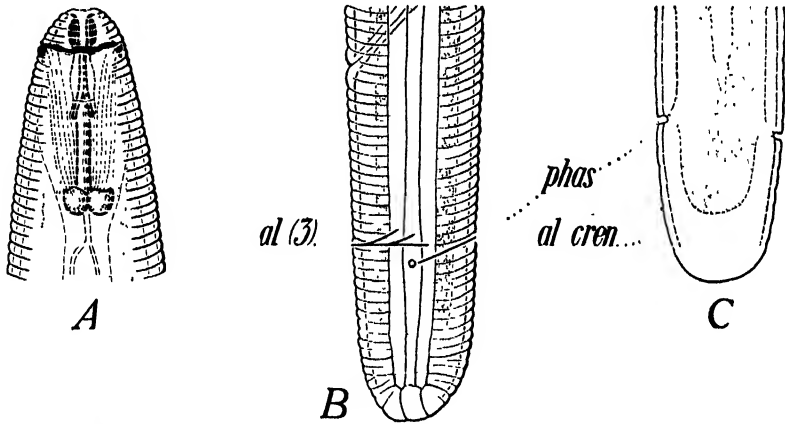


FIGURE 4. *Anguillulina magnicauda*: A, Head. $\times 800$. B, Female tail, lateral view. *al* (3). Wings: *phas*, phasmid. $\times 800$. C, Terminus of female tail, ventral view: *phas*, Phasmid; *al cren*, crenation of wings. $\times 800$.

Measurements.—

3.2	13.	19.	¹⁹ 60. ¹⁷	94.4	
1.4	2.8	3.1	3.	2.4	1 mm

Body almost cylindrical, attaining greatest width at base of esophagus. Anteriorly it tapers moderately until lip region is one-half width of neck at nerve ring. Terminus about hemispherical. Cuticle marked by coarse striae that may, especially on tail, be traced across the wing area. Three wings slightly crenate; wing area occupying about one-third the body width, marked by four refractive lines. Lip region continuous with neck contour, marked by fine striae. Minute amphids and labial papillae visible even from lateral view. Labial framework yellow, massive. Spear length about twice width of lip region, basal bulbs strongly developed. The two sections of spear unusually well differentiated. Median esophageal bulb ovate, half as wide as the neck. Posterior esophageal bulb very definitely differentiated from intestine. Excretory pore opposite middle of posterior bulb. Intestine with variable-sized granules. Rectum shorter than anal body diameter. Anus distinct. Vulva a deep, transverse slit. Vagina extending half way across the body. Amphidelphic ovaries symmetrical, outstretched. Although specimen was well developed, no sperms visible in uterus. Tail almost cylindrical to hemispherical terminus, $2\frac{1}{2}$ times as long as anal body diameter. Unusually large portion of terminus hyaline, the "core" only partially filling it. Phasmids prominent, in profile appearing as distinct pockets with fibrous inner connections leading to the lateral cords.

Diagnosis.—*Anguillulina* with rather cylindrical body, practically straight when killed by gradual heat. Lip region continuous with body contour, half as wide as neck at the nerve ring. Labial framework yellow, massive. Spear with huge basal knobs, its length twice the width of lip region. Two ovaries outstretched, symmetrical. Tail almost cylindrical, $2\frac{1}{2}$ times as long as anal body diameter.

Relationships.—Most closely related to *Anguillulina macrura* Goodey, from which it differs in its much wider lip region, more massive spear, and longer tail. The spear, yellow labial framework, and coarse striae of the cuticle similar to those of *Hoplolaimus*, but lip region not set off and spear knobs not dentate.

APHELENCHOIDES BRACHYCEPHALUS, N. SP.

Small numbers of *Aphelenchoides brachycephalus* (fig. 5, A, B, C, D) were found living in the frass and tunnels of the bark beetle. Among

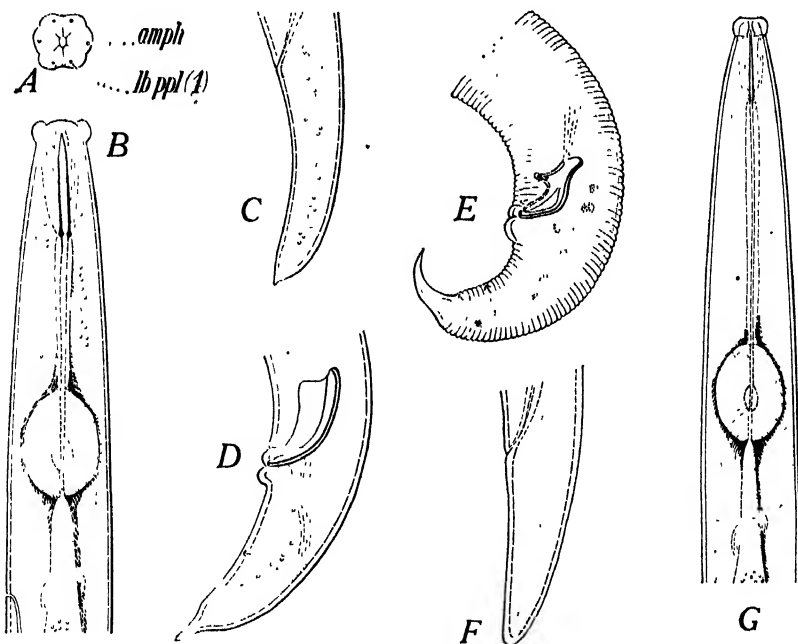


FIGURE 5.—A-D, *Aphelenchoides brachycephalus*. A, Head, face view: amph, Amphid; lb ppl, (l), labial papillae. × 665. B, Anterior end. × 665. C, Female tail. × 665. D, Male tail. × 665. E-G, *Aphelenchoides talonus*. E, Male tail. × 665. F, Female tail. × 665. G, Anterior end. × 665.

the *Aphelenchoides*, *A. brachycephalus* is outstanding because of its broadly expanded, disklike lip region and long, wide spear. The aperture of the spear is easily observed in this species, and it is located ventrally as in other species of *Aphelenchoides* and in *Anguillulina*, *Hoplolaimus*, and related genera. In fact, this feature is a distinctive, previously unrecorded character separating the Anguillulinidae from the Dorylaimidae in which the spear aperture is dorsal.

Measurements.—

2.5	11.	12.	⁵⁷ 76. ¹²	94.	0.8-1 mm
1.3	2.5	2.6	3.	1.7	
2.5	10.	11.	²⁵ M	95.	0.8-1 mm
1.3	2.1	2.2	2.8	2.	

Cuticle finely annulated, about 17 striae lying between the lips and a point opposite the base of the spear. Anteriorly body tapers much less than in other species to the broadly expanded lip region, which is five-eighths as wide as the neck at the bulb. Body ventrally contracted at vulva. Female tail three times as long as anal body diameter, tapering gradually to the abruptly conoid, mucronate terminus. Male tail somewhat shorter than that of female and more definitely mucronate. Face view reveals lateral lips to be much larger than the submedian. Amphids located slightly ventro-submedially on the lateral lips. The four labial papillae shifted dorsally and ventrally. Spear almost twice as long as width of lip region with distinct joint near middle, the aperture occupying one-half of the anterior portion. Anterior portion of esophagus short, only $1\frac{1}{2}$ times spear length. Bulb slightly ovate, three-fourths as wide as body cavity, its musculature indistinct. Nerve ring located about one bulb width behind bulb. Excretory pore near nerve ring. Granules of intestine beginning just back of nerve ring. Ovary outstretched. Eggs twice as long as body width, and as thick as body cavity. Posterior uterine branch extending one-half to three-fourths the distance to the anus. Testis outstretched. Spicula two-fifths as wide as body, arcuate on dorsal side, ventrally consisting of a slender flexible element. Only two pairs of submedian papillae observed, one pair near middle of tail, other slightly preanal.

Diagnosis.—*Aphelenchoides* with lip region five-eighths as wide as neck at bulb. Spear one-sixth as wide and almost twice as long as width of lip region, the aperture occupying one-half of its anterior portion. Body ventrally contracted at vulva. Spicula arcuate on dorsal side, ventrally a flexible element. Tails of both sexes usually mucronate. Associate of mountain pine beetle.

APHELENCHOIDES TALONUS, N. SP.

Many specimens of *Aphelenchoides talonus* appeared in practically all collections. The female is inconspicuous when compared to the male with his "mitten-shaped" spicula and striking, talonlike terminus (fig. 5, E, F, G).

Measurements.—

1.4	10.	12.	⁵⁰ 73. ¹²	96.	0.8 mm
0.7	2.	2.1	3.	1.5	
1.4	9.	10.	⁷⁵ M	98.	0.8 mm
0.7	1.9	2.	2.1	2.	

Anteriorly body is slightly convex-conoid to the amalgamated, truncate, definitely set-off lip region, which is one-third as wide as the neck at the bulb. Female tail convex-conoid to the blunt, rounded terminus, which bears no mucro. Male tail ventrally arcuate, ending in cuticular, talonlike terminus. Spear slender, without basal knobs, its length equal to twice the width of the lip region. Vulva with slightly elevated labia. Ovary extending forward, then reflexed a short distance. Posterior uterine branch reaching three-fourths the way to the anus. Eggs 2 to $2\frac{1}{2}$ times as long as body width. Testis reflexed a short distance.

Diagnosis.—*Aphelenchoides* with above measurements. Male with "mitten-shaped" spicula and cuticular talonlike terminus. Female tail conoid to the blunt rounded terminus, which bears no mucro. Lip region amalgamated, definitely set off. Spear without basal knobs, its length equal to twice width of lip region. Associate of mountain pine beetle.

APHELENCHOIDES TENUIDENS, N. SP.

Many larvae of *Aphelenchoides tenuidens* (fig. 6) held together in masses of cocoonlike material were found under the elytra of several beetles. Large numbers of the adults were in the bodies of dead beetles and in the frass from the tunnels. In no instance were they found within the bodies of living insects.

Measurements.—

2.1	11.	13.	⁵⁵ 75. ¹⁵	95.	0.8 mm
1.	2.1	2.3	2.7	1.7	
2.2	12.	14.	⁶⁷ M	94.5	0.75 mm
1.1	2.2	2.3	2.6	2.2	

Body tapering rapidly anteriorly, the width at esophageal bulb being $2\frac{1}{2}$ times that of lip region. Female tail 3 times as long as anal body diameter, slightly convex-conoid to the abruptly conoid terminus, which does not bear a distinct mucro. Male tail slightly bent ventrally, terminus mucronate. Four pairs of submedian papillae present, 2 pairs preanal and 2 caudal (fig. 6, C). Spicula about two-fifths as wide as body, arcuate in distal half, proximally almost straight on dorsal side; ventral side flexible when spicula are extruded (fig. 6, D).

The distinct striations of the cuticle interrupted laterally by a wing area, which near middle of nema is about one-eighth as wide as body. Lip region amalgamated, caplike, set off by constriction. Vestibule well cuticularized. Spear very slender, its length almost twice the width of lip region, with obscure basal swellings. In living specimens distinct joint observed near middle of spear. Esophageal bulb ovate, with strong musculature, a little more than half as wide as neck: Nerve ring one bulb length behind bulb. Excretory pores slightly back of nerve ring. Intestines densely granular. Ovary outstretched, sometimes almost reaching esophagus. Posterior uterine branch reaching one-half to three-fourths the distance to anus. Eggs half as wide as body; $2\frac{1}{2}$ times as long as wide. Testis usually outstretched, occasionally reflexed a short distance.

Diagnosis.—*Aphelenchoides*.—Spear twice as long as width of lip region, with obscure basal swellings. Male terminus with mucro, female terminus without mucro. Spicula two-fifths as wide as body, proximally almost straight on dorsal side, ventrally slender, flexible. Male caudal papillae arranged as shown in figure 6, C. Associate of mountain pine beetle.

APHELENCHOIDES LATUS, N. SP.

Aphelenchoides latus (fig. 7) is very closely related to *Aphelenchoides macrogaster* (Fuchs, 1915) new comb. (synonym, *Tylenchus macrogaster* Fuchs, 1915),⁸ from which it differs principally in the narrower lip region and more conoid tails.

Measurements.—

2.5	16.	19.	W	⁴⁰	80.	92.5	0.4 mm
1.5	4.4	4.5	5.5		5.	3.3	
2.7	18.	21.	"	M	92.3	0.4 mm	
1.7	4.4	4.5	5.		3.7		

Body short, unusually broad for an *Aphelenchoides*, tapering anteriorly until lip region is about two-fifths as wide as the neck at the bulb. Wing area marked by four lines. Annulation broad, obscure. Body slightly contracted ventrally at vulva. Female tail slightly arcuate, conoid to pointed terminus. Male tail ventrally arcuate, conoid to pointed terminus. Lip region set off by slight depression, lips obscure. Spear with well-developed basal knobs. Esophageal bulb very large. Hyaline esophagus extends back from bulb unusually long distance before merging with intestine (fig. 7, A). Excretory pore located about opposite first granules of intestine. Granules of intestine and body generally large. Vulva a depressed transverse slit, anterior lip overlapping. Vagina at

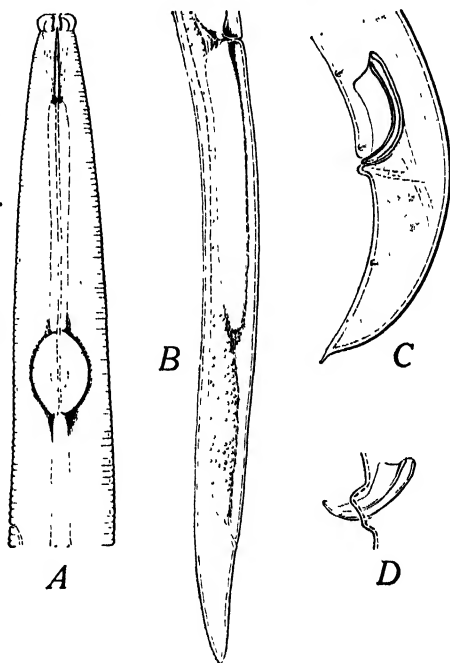


FIGURE 6.—*Aphelenchoides tenuidens*: A, Anterior end. $\times 665$. B, Posterior portion of female. $\times 335$. C, Male tail. $\times 665$. D, Spicula partly extruded. $\times 665$.

⁸ FUCHS, G. See footnote 6.

first extending in and forward, then bent to nearly right angles with body axis. Ovary outstretched. Posterior uterine branch extending almost to rectum. Female rectum and anus inconspicuous. Two pairs of conspicuous, conical male papillae, one slightly preanal and other at beginning of distal third of tail. Spicula elongate, mitten-shaped, cephalated (fig. 7, B). Sex ratio, about eight females to each male.

Diagnosis.—*Aphelenchoides* of small size, and broad body with above measurements. Spear with basal knobs. Esophageal bulb comparatively massive. Intestinal granules beginning almost two body-widths behind bulb. Excretory pore about opposite anterior end of intestine. Body slightly contracted at vulva. Spicula elongate mitten-shaped. Tail of female slightly arcuate, that of male conspicuously arcuate. Terminus acute. Beneath elytra and in tunnels of mountain pine beetle.

DIPLOGASTER PINICOLA, N. SP.

Diplogaster pinicola (fig. 8) was present in limited numbers, which may have been due to the fact that many individuals were suffering

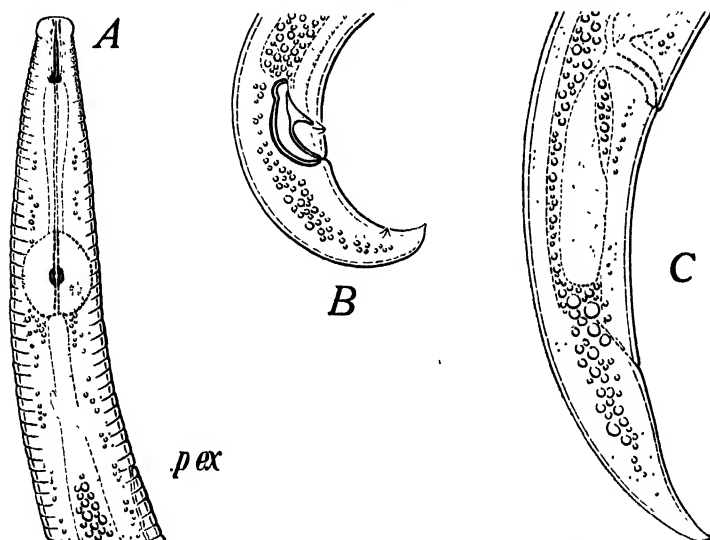


FIGURE 7.—*Aphelenchoides latus*. A, Anterior end: p ex, Excretory pore. $\times 800$. B, Male tail. $\times 800$. C, Posterior portion of female. $\times 800$.

from a cutaneous affection, apparently bacterial, causing blisterlike elevations on the head and neck. The dentition indicates that the species may be predaceous, but identifiable food particles were not present in the intestine. The species closely resembles *Diplogaster butschlii* Fuchs, 1915, from which it differs in possessing longitudinal striations of the cuticle and in the pattern of the cuticular markings.

Measurements.—

0.6	9.	14.	²⁰ 51.	²² 93.4	1.3 mm
1.5	2.4	2.7	3.9	1.9	
0.8	12.	16.	⁵² M	93.6	1.1 mm
1.2	2.	2.4	3.2	2.8	

Body moderately slender, tapering anteriorly until width near lip region is about one-half that at base of neck. Female tail convex-conoid to acute terminus, its length about $2\frac{1}{2}$ times anal body diameter. Male tail ventrally arcuate, convex-conoid with spicate terminus. Cuticle marked by fine transverse and longitudinal striae. Longitudinal striae low, obscure (fig. 8, B), about 44 at mid body,

decreasing in number toward the extremities. Viewed laterally these longitudinal striae present double rows of refractive, dotlike markings where they cross the transverse striae (fig. 8, C). Lip region rounded, with six forward-pointing, conical papillae. Amphids appear as minute oval markings close to the lateral papillae. Pharynx obscurely hexagonal from a face view; viewed laterally it presents two distinct chambers (fig. 8, D) bearing a central, massive, arcuate, dorsal tooth. Anterior portion of esophagus four-fifths as long as posterior but broader and more muscular (fig. 8, A). Excretory pore a short distance posterior to nerve ring. Intestine densely granular, its lumen sinuous. Ovaries reflexed past vulva. Vulva a transverse slit with protuberant labia. Testis single, reflexed. Spicula yellow, arcuate, slightly cephalated. Gubernaculum thick proximally, with a

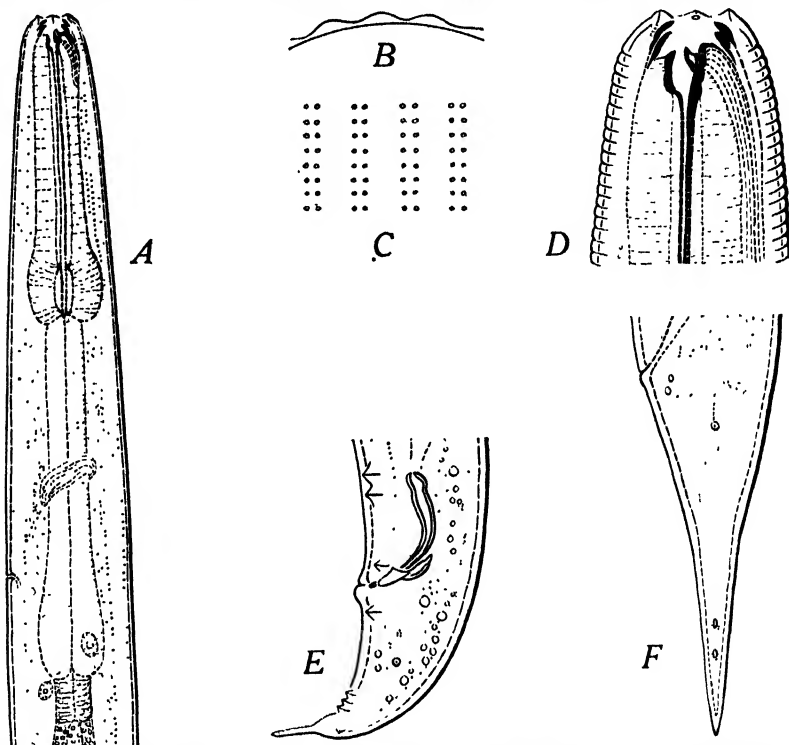


FIGURE 8.—*Diplogaster pinicola*: A, Anterior end. $\times 500$. B, Cross section through three longitudinal striae of cuticle. $\times 2,000$. C, Pattern formed by dotlike markings of cuticle. $\times 2,000$. D, Head. $\times 1,000$. E, Male tail. $\times 500$. F, Female tail. $\times 500$.

thin troughlike distal extension in which the spicula glide. Eight pairs of male caudal papillae (fig. 8, E).

Diagnosis.—*Diplogaster* with the above measurements. Longitudinal striae 44 at mid body, low, obscure, their presence indicated by double rows of refractive dots. Tails of both sexes less than 7 percent of body length. Six labial papillae, forward-pointing, conical. Pharynx divided into two chambers, armed with single, massive, arcuate dorsal onchium. Female amphidelphic, ovaries reflexed past vulva. Spicula arcuate, cephalated. Gubernaculum thick proximally with thin troughlike distal extension. Eight pairs of male caudal papillae (fig. 8, E). From frass and tunnels of mountain pine beetle.

RHABDITIS OBTUSA FUCHS, 1915

Hundreds of the nema *Rhabditis obtusa* (fig. 9) were found in a single tree in which "sour sap" had followed beetle infestation. Fuchs⁹ described varieties of *R. obtusa* based principally on the bursal

⁹ FUCHS, B. See footnote 6.

formula. This character was variable on the specimens examined and definite varieties could not be determined. In fact, it is doubtful whether the varieties proposed by Fuchs are as definite as he thought them to be.

Measurements.—

2.3	13.	20.	W	⁶⁴ 95.	98.2	0.8—1.1 mm
2.1	3.	3.6	4.	4.	2.1	
2.6	14.	22.	⁶⁰ M	95.6		0.6—0.8 mm
2.2	2.7	3.3	3.4	3.3		

Bodies of both sexes almost cylindrical between esophagus and genital opening. Neck tapering uniformly to lip region, which is about one-third as wide as base of neck. Female tail short, bluntly conoid. Vulva exceedingly far back (fig. 9, A). Striae about 1μ wide at mid body, slightly wider near head. Lip region almost continuous with neck contour. Six conical, forward-pointing, labial papillae were visible but other details of head were always obscured by clinging debris. Amphids minute. Pharynx about three times as deep as wide. Cheilorhabdions and protorhabdions slightly convex. Telostom absent. Esophagus: Corpus

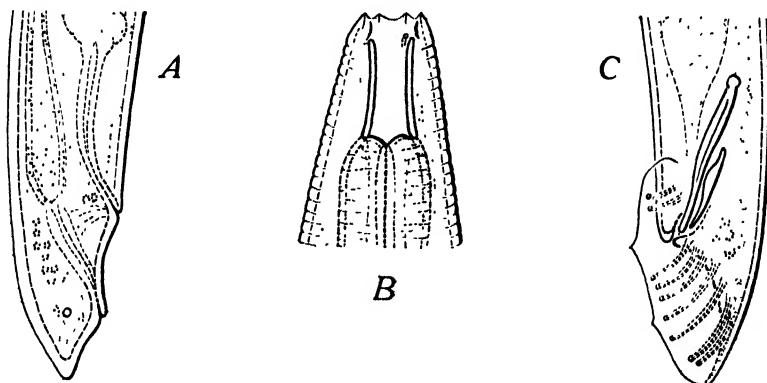


FIGURE 9.—*Rhabditis obtusa* Fuchs: A, Posterior portion of female. $\times 500$. B, Head. $\times 1,000$. C, Male tail. $\times 500$.

cylindrical; medial bulb slightly wider than corpus; isthmus same length as corpus and half as wide; terminal bulb ovate, two-thirds as wide as neck. Nerve ring midway of isthmus. Excretory pore slightly posterior to nerve ring. Female prodelphic. Vulva elevated. Vagina extending almost straight forward. Uterus one-third as long as body. Ovary extending forward from uterus, then reflexed until the blind end reaches one-half to three-fourths the distance back to the vulva. Posterior uterine branch absent. Testis single, extending nearly to esophagus, then reflexed a short distance. Spicula and gubernaculum as shown in figure 9, C. Bursa enveloping the tail, with 2 pairs of preanal ribs, then 3 pairs grouped close together just posterior to anus, followed by 4, rarely 3 or 5, pairs; general bursal formula being 2(0)3, 1, 1, 2.

The species differs from *Rhabditis lambdiensis* Maupas in its slenderer body, continuous lip region, absence of telostom, form of spicula and gubernaculum, bursal formula, and bluntly conoid female tail. It is distinguished from *R. monkhystera* Bütschli by the bluntly conoid female tail, by the short male tail enveloped by the bursa, and by the bursal formula.

PANAGRODONTUS, N. G.

Diagnosis.—Cephalobidae. Cephaloboid nemas bearing a flat toothlike projection on the dorsal prorhabdion, which may be opposed by submedian onchia. Lips three, duplex. Amphids minute. Corpus of esophagus broad, cylindrical.

Ovary single, extending forward from the vulva, then reflexed far posterior to it, lacking the double flexure found in *Acrobeles* and *Cephalobus*, the blind end usually reaching the rectum and frequently extending past the anus into the tail. Testis single, terminal portion reflexed. Spicula equal, arcuate, cephalated. Gubernaculum present.

Type species.—*Panagrodontus dentatus*, n. sp.

Panagrodontus differs from other Cephalobidae in possessing a dorsal pharyngeal onchium which sometimes is opposed by submedian onchia.

PANAGRODONTUS DENTATUS, N. G., N. SP.

Measurements.—

1.6	12.	20.	W	³⁰ 59. ³⁰	90.	0.6 mm
1.9	4.2	4.6	5.6	4.9	3.	
1.3	11.	18.	⁶⁰ M	90.	0.6 mm	
1.6	3.6	4.2	4.2	3.6		

Body tapering both ways from near middle. Tails of both sexes at first dorsally convex-conoid, then convex, ending in a somewhat spicate terminus which occupies one-third to one-half of the total tail length. Transverse striae moder-

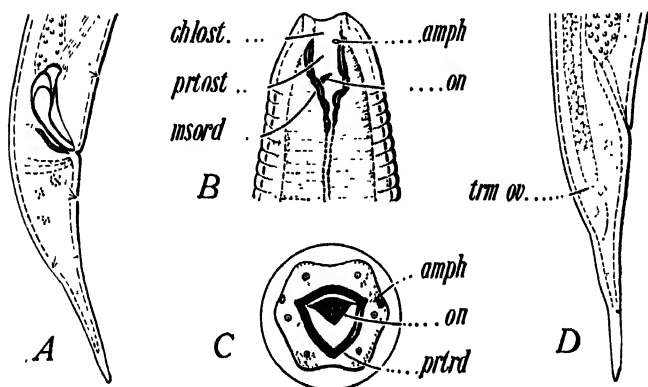


FIGURE 10.—*Panagrodontus dentatus*. A, Male tail. $\times 500$. B, Head, lateral view: chlost., Cheilostome, prtost., protostome; msord., mesorhabdion; amph., amphid; on, tooth. $\times 1,000$. C, Head, face view: amph., Amphid; on, tooth; prtrd., protorhabdion. $\times 2,000$. D, Female tail: trm ov., Terminus of ovary. $\times 500$.

ately fine. Wings two, obscure, the area about one-eighth as wide as body near the middle. Lip region rounded, continuous with neck contour. Lips three, duplex, the two subventral being somewhat asymmetrical. Amphids minute. Pharynx:¹⁰ Cheilostome obscure, hexagonal when seen in face view; protostome triquetrous; dorsal mesorhabdion bearing a flat toothlike plate about 2μ long opposed by a niche formed by the submedian mesorhabdions and metarhabdions. Esophagus: Corpus cylindrical, at first almost filling body cavity; isthmus about equal in length to corpus; bulb half as wide as neck with conspicuous valvular apparatus. Intestinal walls at first thin then gradually becoming much thicker with a corresponding narrowing of the lumen. Vulva a transverse slit with elevated labia. Posterior uterine branch rudimentary, its length equal to 1 or 2 body-widths. Ovary extending forward, then reflexed and outstretched, the terminus reaching the rectum or, frequently, extending into the tail. Average size of eggs $20\mu \times 50\mu$. Testis single, the terminal portion reflexed. Spicula, gubernaculum, and male caudal papillae as shown in figure 10, A.

Diagnosis.—*Panagrodontus* with above measurements. Pharynx armed with a single tooth located on the dorsal mesorhabdion. Tails of sexes similar, at first dorsally convex-conoid, then convex, ending in a somewhat spicate terminus. Spicula, gubernaculum, and male caudal papillae as shown in figure 10, A. Associate of mountain pine beetle.

¹⁰ STEINER, G. NOMENCLATURE OF PHARYNGEAL PLATES IN THE NEMATODE CYLINDROGASTER LONGISTOMA (STEFANSKI) GOODEY, AND ITS RELATIONSHIP. Jour. Parasitol. 20: 66. 1933.

SUMMARY

A brief historical discussion of the mountain pine beetle in Utah is given.

A new nemic endoparasite, *Aphelenchulus reversus*, is described and its life history partially outlined.

Eight ectoparasites and associates new to science are described: *Anguillulina pinophila*, *A. magnicauda*, *Aphelenchoides brachycephalus*, *A. talonus*, *A. tenuidens*, *A. latus*, *Panagrodontus dentatus*, and *Diplogaster pinicola*.

New information is given concerning *Rhabditis obtusa* Fuchs, 1915.

Phases of the life histories and habits of these nemas are discussed.

A diagnosis of the new genus *Panagrodontus* is made.

STUDIES ON THE VARIABILITY OF PATHOGENICITY AND CULTURAL CHARACTERS OF *GIBBERELLA SAUBINETII*¹

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INTRODUCTION

Variability in pathogenicity and cultural characters in the fungus *Gibberella saubinetii* (Mont.) Sacc. was brought to the attention of the writer during the course of an investigation on the influence of soil temperature upon the development of seedling blight in barley (*Hordeum vulgare* L.) caused by this organism. As a result of these observations the present investigation was directed toward determining the nature, frequency, and magnitude of the apparent variability in *G. saubinetii*, particularly with respect to pathogenicity and cultural characters.

MATERIAL AND METHODS

Perithecia of *Gibberella saubinetii* collected by the writer in the summer of 1933 from barley fields in Illinois, Iowa, and Minnesota furnished the material on which this study was based. All cultures used were grown from ascospores isolated in sets of 8, each set comprising the 8 ascospores from a single ascus. Each subsequent subculture, however, was derived from a single conidium unless otherwise indicated. Ascospores, rather than conidia, were selected as starting points because, theoretically, the ascospores should be homocaryotic, whereas the conidia may or may not be in that condition. Granting the possibility of the existence of nuclei of different genetic constitution, a random assortment of nuclei by virtue of the frequently occurring phenomenon of anastomosis would not preclude the possibility of conidia of different nuclear make-up.

The procedure employed in the isolation of the ascospores was to strip off a bit of the epidermis of a cornstalk bearing the perithecia and wash it in several changes of sterile distilled water. Then it was placed in a sterile Petri dish and the excess water allowed to evaporate. The dish with cover removed was placed under a dissecting microscope and an individual perithecium was picked off with a small flattened needle and placed in a drop of distilled water on a flamed microscope slide. The perithecium was then crushed between the slide and a flamed cover slip. The asci and spores thus removed

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² The writer acknowledges his indebtedness to J. G. Dickson, professor of plant pathology, Wisconsin Agricultural Experiment Station, under whose direction this work was undertaken, and to J. C. Walker, professor of plant pathology, Wisconsin Agricultural Experiment Station, and Helen Johann, associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful criticism of the manuscript.

from the confines of the fruiting body were observed under the microscope to determine their stage of maturity. Experience has shown that apparently there is a rather definite stage in the development of the asci and ascospores before or after which successful isolation of a set of eight spores from a single ascus is impossible. If the spores are immature, although they may be well defined within the ascus, they are difficult to remove. Furthermore, germination of the spores either does not take place or is at best very weak. If, on the other hand, the contents of the perithecium have advanced beyond the desirable stage of development, the spores are liberated from the ascus while the latter is still within the perithecium, thus making it impossible to isolate the spores with regard to the ascus in which they were borne.

Spores and asci that had attained the proper stage of maturity, as determined by observation, were picked up with a fine wire loop along with the water in which they were suspended, and the suspension was streaked over the surface of hard 4-percent water agar contained in Petri dishes. After about 5 hours' incubation at 24° C., the spores within the asci showed germ tubes, approximately one-half the length of the spore, protruding through the ascus wall. With the aid of the low power of the microscope the locations of asci bearing eight germinating ascospores were marked by scarifying the surface of the agar adjacent to each ascus. When a sufficient number of asci had thus been located, the agar plate was placed under a dissecting microscope, and each ascus was moved across the surface of the agar to an area free from spores and fragments of the perithecial wall. With the asci thus removed from extraneous material and separated at sufficient distances from each other, the spores were "teased" out of each ascus and separated from one another at convenient distances within the field of vision. All manipulations, including the separation of the asci from spores and other material and the removal of spores from the asci, were done by hand with the aid of a sterilized fine glass wire 2 μ to 3 μ in diameter and bent to form a right angle or semiloop. After the spores were removed from the asci and separated from one another they were examined under the microscope and then allowed to continue germination for a period of about 3 hours. By means of a small loop cutter 0.5 mm in diameter, a cylinder of agar was cut around each spore and the entire piece of agar with the spore on its surface was lifted out and placed in the center of a Petri dish containing hard potato-dextrose agar. Each plate was placed under a microscope and again examined to make certain that only a single ascospore had been transferred.

Hyphal-tip cultures were obtained by the same general method as that just described for single ascosporic cultures. After the eight spores from a single ascus had been separated from one another, they were allowed to continue germination until the germ tubes were 5 to 6 times the length of the spore. By means of a fine sterilized glass needle a light scratch was made across the tip of the germ tube, back of a septum, severing it from the spore proper. The free hyphal tip was picked up and placed in the center of a Petri dish containing hard potato-dextrose agar. Two hyphal tips were thus cut off from each spore and grown in separate Petri dishes.

A system of numbering was devised whereby the cultures and culture sets just described could be designated. In this system a number was given to each culture that would indicate the State, the location in the State where the collection was made, the perithecium, the ascus from which the eight spores were isolated, and finally the single ascospores or hyphal tip from which the culture was grown. In table 1 are given the culture sets used, together with the location where the original perithecial material was collected and the date on which the isolation was made.

TABLE 1.—*Cultures used in the investigations reported in this paper, together with the origin of the perithecial material and the date of isolation*

Culture no.	Origin of perithecial material	Date of isolation	Culture no.	Origin of perithecial material	Date of isolation
		1933			1933
Ill. 111-1 to 7.....	Mineral, Ill.....	July 14	Minn. 111-1 to 8...	Olivia, Minn.....	July 17
Ill. 121-II, T ¹	do.....	Do.	Minn. 112-II, T.....	do.....	Do.
Ill. 132-H T.....	do.....	Oct. 15	Minn. 211-1 to 8...	Alpha, Minn.....	Nov. 2
Iowa 111-1 to 8.....	Calumet, Iowa.....	July 14	Minn. 212-1 to 8...	do.....	Do.
Iowa 211-1 to 8.....	West Liberty, Iowa.	July 28			
Iowa 221-1 to 8.....	do.....	Oct. 15			

¹ Hyphal tip

CULTURAL STUDIES

In order to obtain some information as to the range, type, and factors influencing variability in cultural characters, four different experiments were set up. In these experiments potato-dextrose agar and a modification of Brown's agar were used. Each subculture studied was grown from a single conidium unless otherwise indicated. Isolations of single conidia were made by streaking a spore suspension over the surface of hard agar and allowing the spores to germinate. After germination had commenced the spores were picked up singly and placed on the particular agar medium on which they were to be grown. The quantity of agar used in the Petri dishes in which the single-spore colonies were grown was approximately 20 cc. The colonies were allowed to develop in the dark at room temperature, which varied between 21° and 23° C. At the end of 5 days, except in case of first isolation, descriptive notes were taken on each culture with respect to color, type of growth, diameter of colony, type of margin, and the expression of zonation and radiation. Although it is admitted that descriptive notes do not adequately convey a picture of the real differences and changes that occurred in the cultures, nevertheless, such notes, together with photographs, are of value in recording the major variations in cultural behavior.

The following is a brief description of the original colonies grown at room temperature for a period of 4 days:

Color of colony, pomegranate purple³ concentrated in a central area 2 to 3 cm in diameter and gradually fading to white toward the edge of the colony. Aerial mycelium abundant, extending over the entire colony, and of a cottony texture. Margin of colony, generally regular and entire. No pronounced zonation or radiation detectable. Colony diameter, 8 cm.

³ RIDGWAY, R. COLOR STANDARDS AND NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912

The foregoing description applies to all of the original isolates, whether grown from ascospores or hyphal tips (figs. 1 and 2) and regardless of their geographical origin. These isolates constitute a

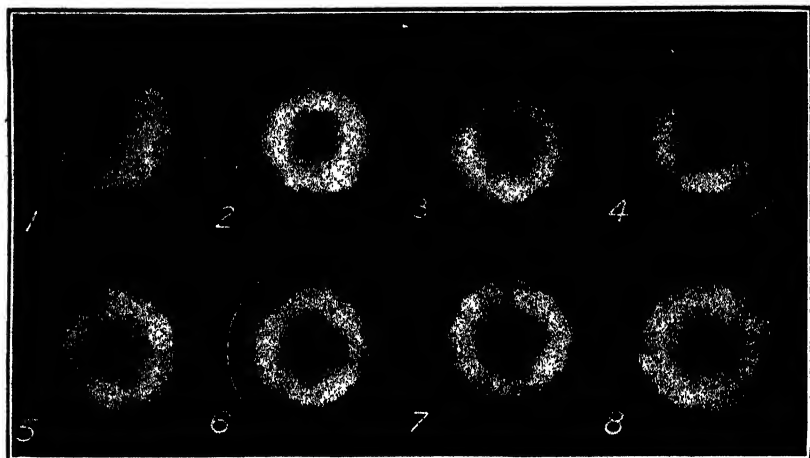


FIGURE 1.—Eight single ascosporic colonies at 4 days of age, grown from the eight ascospores of a single ascus.

type always encountered on first isolation and referred to in this paper as "type A."

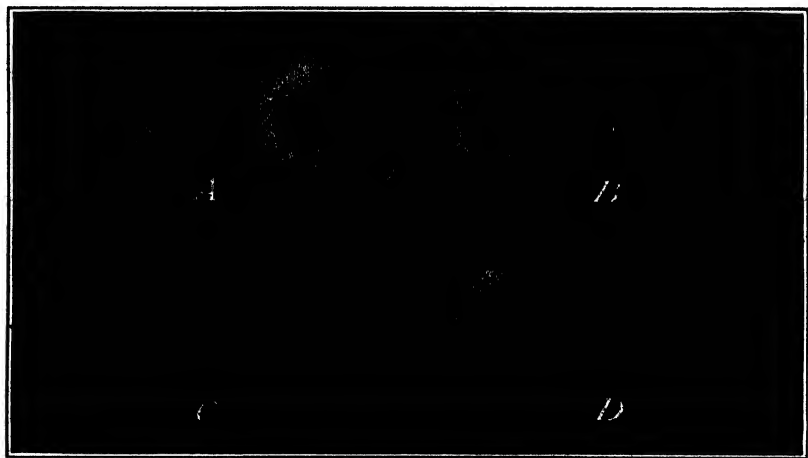


FIGURE 2.—Four pairs (A-D) of hyphal-tip colonies at 4 days of age. The two members of a pair were grown, respectively, from hyphal tips arising from opposite ends of a germinating ascospore.

EXPERIMENT 1

Experiment 1 was designed to study the effect of the medium and the cultural technic on the changes in cultural characters. Eight single ascosporic cultures from an individual ascus were employed in the experiment. The eight original colonies were strikingly similar in appearance (fig. 1). Duplicate mass transfers were made from each

of the colonies, one transfer to a slant of potato-dextrose agar and the other to a slant of modified Brown's agar. Three days later, spore suspensions were made in these culture tubes and single conidial isolations were made in triplicate from each culture. Part of the spore suspension was used to seed fresh slants of agar from which single conidial isolations were again made 3 days later. This procedure of subculturing from slant cultures that had been transferred every 3 days was repeated 28 times.

During the course of the experiment several of the cultures showed marked changes in cultural behavior from that of the original ascospore culture. In the cultures propagated on potato-dextrose agar, nos. 1, 2, 4, 6, and 7 were strikingly different by the end of the experiment, while nos. 3, 5, and 8 remained constant (fig. 3). Of the cultures grown on modified Brown's agar, only no. 7 maintained its

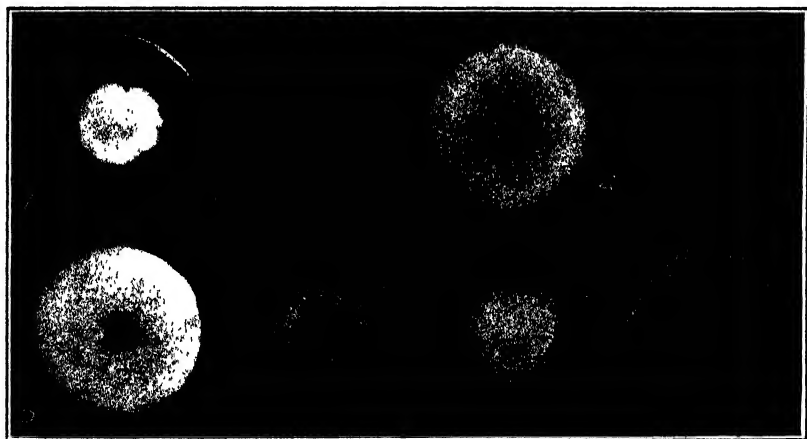


FIGURE 3.—Eight monoconidial colonies after having been subcultured 28 times on potato-dextrose agar. Originally, these cultures were all similar in appearance and were derived from the eight ascospores of a single ascus. Nos. 1, 2, 4, 6, and 7 were strikingly different at end of experiment. Nos. 3, 5, and 8 remained constant.

original appearance, the others having changed decidedly in cultural behavior (fig. 4).

It is apparent that the medium on which the cultures were grown had no influence in determining the extent and magnitude of variation that occurred in cultural characters. Duplicate cultures, each on a separate medium, did not behave in a manner that would indicate their relationship. If the capacity or tendency of a culture to vary in its behavior while growing under artificial conditions is associated with its genetic constitution, then it is not unreasonable to expect cultures arising from the same original ascospore colony to behave in a similar manner unless irregularities of one sort or another have occurred. Under the conditions of the experiment, in which considerable care was taken to maintain the cultures at a constant environment, there seemed to be no uniformity in the changes that the cultures underwent, regardless of their origin or the media used. The variant cultural types that appeared were generally characterized by a slower radial growth rate and a decrease in abundance of aerial mycelium.

EXPERIMENT 2

The primary object of experiment 2 was to determine what influence the frequency of transfer might have on the cultural behavior of a set of eight single ascosporic isolates from the same ascus.

Duplicate transfers were made from the eight original colonies, which were essentially alike in appearance, to slants on modified Brown's agar. One of these sets of slant cultures was subsequently transferred every 4 days, the other set every 8 days. Each time the cultures were transferred single conidial isolations were made in triplicate from each isolate.

During the course of the experiment the majority of isolates changed in cultural appearance. There was apparently no difference in behavior between the isolates of the group transferred every 4 days and those transferred every 8 days. The time elapsing before the changes became evident and the extent and the magnitude of these

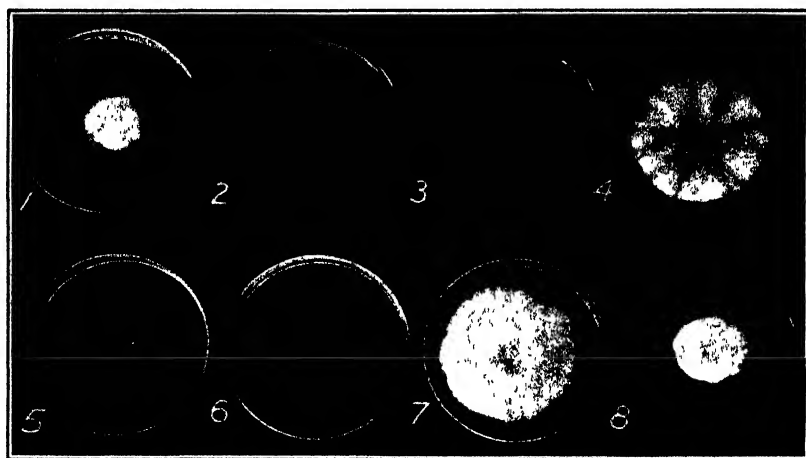


FIGURE 4.—Duplicates of colonies shown in figure 3 and originally derived from the same ascus. They were subcultured 28 times on modified Brown's agar. Nos. 1 to 6 and 8 changed in cultural behavior. No. 7 remained constant.

changes in cultural type were essentially the same in both groups. The results from this experiment, like those from experiment 1, seemed to indicate that changes in cultural characters were not of a systematic, genetic nature.

EXPERIMENT 3

Studies were made to determine the effect of continued subculturing on the stability of a set of eight single ascosporic isolates from an individual ascus. In experiment 3 the isolates were propagated only in Petri dishes instead of in culture tubes, as in experiments 1 and 2. As in the other experiments, the original ascosporic colonies were alike in cultural appearance. When the original ascosporic colonies were 6 days old a spore suspension was made in each dish containing a colony, and from each, single conidial isolations were made in triplicate. Although this procedure was continued for 10 successive conidial generations, no changes in cultural behavior became evident. Each of the 8 cultures was now transferred to a slant of modified Brown's

agar and subsequent transfers were made every 6 days. At each time of transfer single conidial isolations were made in triplicate from each of the cultures. After the second transfer on agar slants, changes in cultural characters began to appear in two of the isolates. The variant cultural types were characterized by a reduced growth rate and a decrease in aerial mycelium.

EXPERIMENT 4

The object of experiment 4 was to determine what effect continued subculturing might have on single cultures. Only 2 cultures were

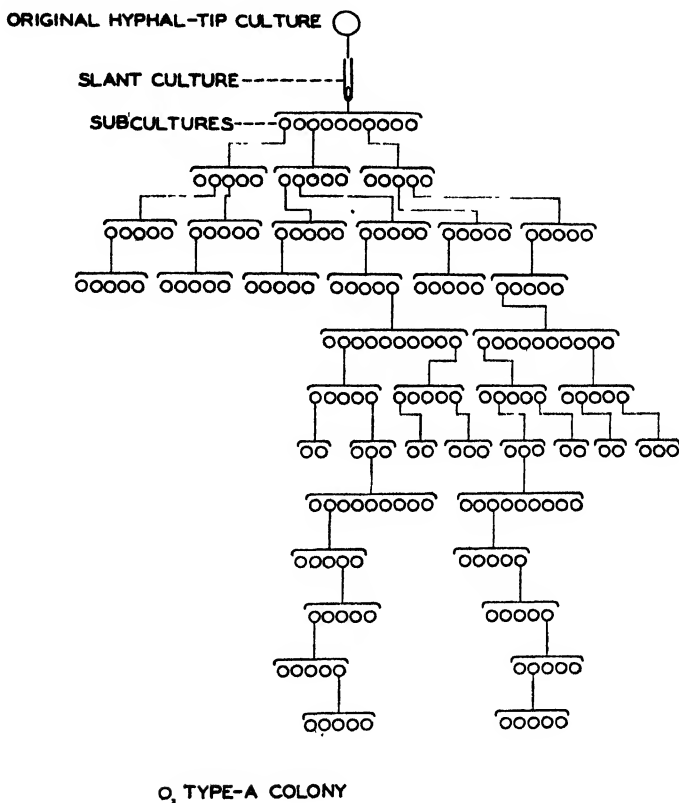
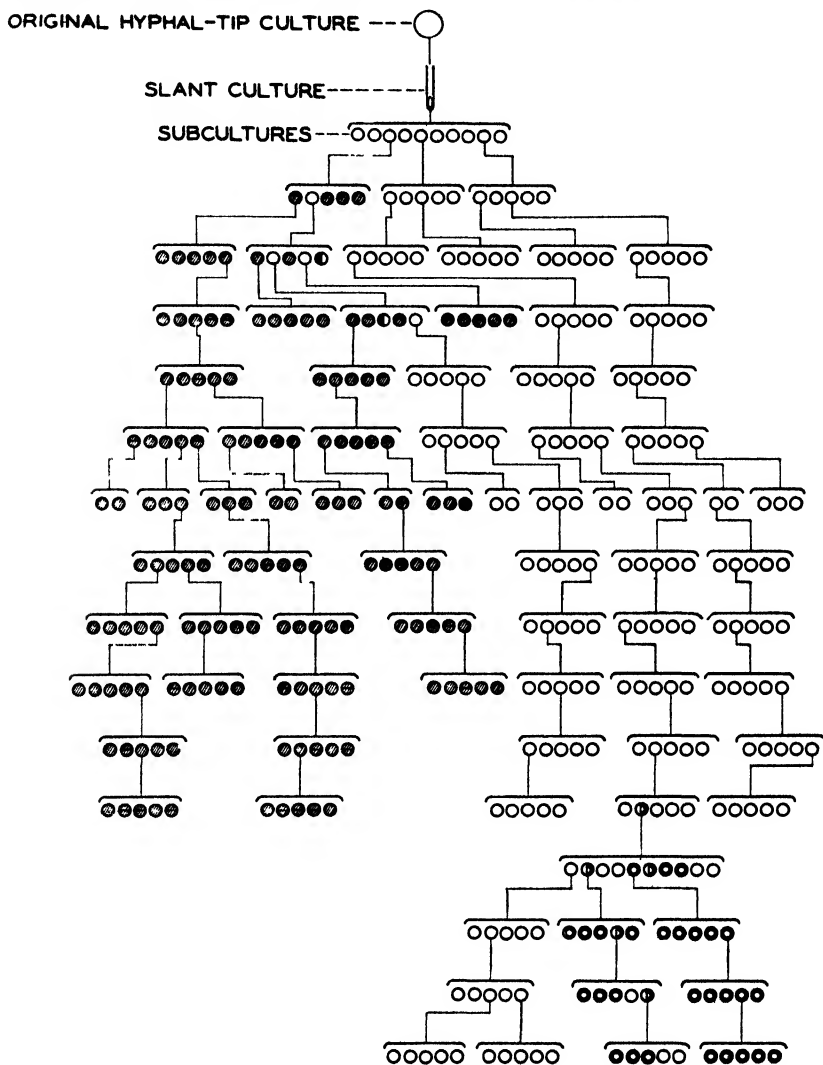


FIGURE 5.—Diagram of procedure followed in experiment 4 in the use of culture Ill. 132-1A. Each circle represents a colony of monoconidial origin isolated from a colony of the previous conidial generation.

studied in detail, namely, Ill. 132-1A and Ill. 132-1B. These were hyphal-tip cultures from a single ascospore and, on original isolation, were exactly alike in cultural behavior. From each of these 2 original cultures, 10 single conidial isolations were made and plated out on hard potato-dextrose agar. After these 20 cultures were 6 days old, single conidial isolations were again made from some colonies of the previous conidial generation. This procedure was continued for more than 16 conidial generations in the culture Ill. 132-1B and 12 conidial generations in the culture Ill. 132-1A (figs. 5, 6).

In the first conidial generation of culture Ill. 132-1B all of the 10 colonies were alike and similar to the original parent colony, that



- , TYPE-A COLONY
- ◐, TYPE-A COLONY WITH TYPE-B SECTOR
- ◑, TYPE-B COLONY
- ◒, TYPE-B COLONY WITH LIGHT SECTOR
- ◓, TYPE-C COLONY
- ◔, TYPE-A COLONY WITH TYPE-C SECTOR

FIGURE 6.—Diagram of procedure used in experiment 4, in which culture Ill. 132-1B was employed. Each circle represents a colony of monoconidial origin isolated from a colony of the previous conidial generation.

is, type A. In the second conidial generation 5 spores selected from one of these colonies produced 5 colonies, 3 of which were alike but

differed distinctly from the parent colony in color, rate of growth, and type of growth. This cultural type will be referred to as type B. The type-B colony, grown at room temperature for a period of 5 days, may be described as follows:

Color of colony, pomegranate purple⁴ in central area 0.5 cm in diameter, surrounded by yellow olivaceous zone that fades to pale yellow at edge of colony. Aerial mycelium scant and extending to 1 cm from edge of colony. Margin regular and entire. Color zones as described, radiations in yellow olivaceous zone. Diameter of colony, 5 cm.

The fourth colony was like the parent colony, and the fifth, although resembling the parent colony in the main, had a small sector resembling the type-B colony (fig. 7). Subsequent isolations from type-A colonies of this line produced type A, type B, and type A with type-B sectors. Further isolations from type-B colonies always

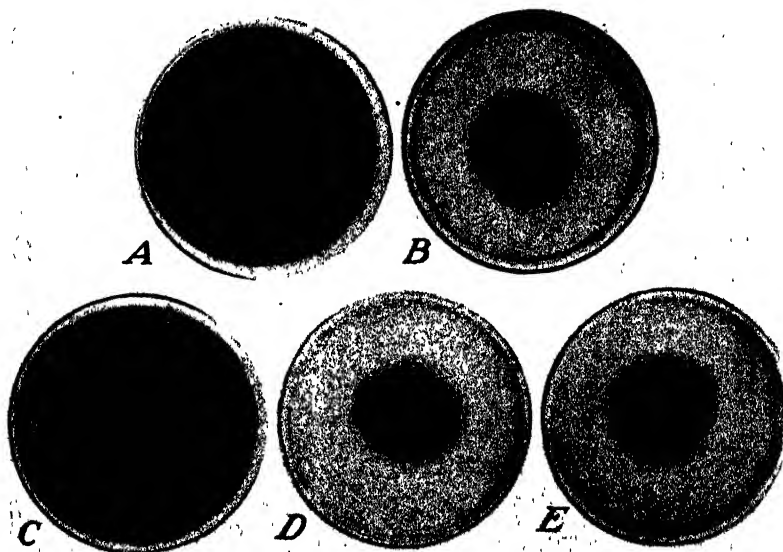


FIGURE 7.—Five single conidial colonies at 5 days of age, all of which were isolated from a type-A colony. A, Colony of type A; B, D, E, three colonies of type B; C, colony of type A with a type-B sector.

gave rise to colonies resembling the type-B parent, although frequently, after the colonies were 5 days old, light-colored sectors appeared in some of these type-B colonies.

The type-A colonies from the other lines remained constant until the twelfth conidial generation, when one colony produced a type-C sector. Isolations from this colony gave rise to colonies of type C, type A, and type A with type-C sectors. Subsequent type-A colonies remained constant, as did the type-C colonies. The following is a description of the type-C colony:

Color of colony, spinel pink⁴ in central area surrounded by narrow zone of pomegranate purple,⁴ which, in turn, was surrounded by a zone of spinel pink, which faded gradually to white at edge of colony. Aerial mycelium abundant, extensive, and of a dense cottony texture. Margin regular and entire. Color zonation as indicated. Radiations in spinel-pink zone. Diameter of colony, 6.5 cm.

⁴ RIDGWAY, R. See footnote 3.

The culture Ill. 132-1A was studied parallel with Ill. 132-1B. During the course of 12 conidial generations no deviations from the original cultural type were found, that is, all colonies were of type A throughout the experiment.

In this experiment, as well as in the experiments reported earlier in this paper, the results appear to lend themselves to 1 of 2 possible interpretations: (1) Variations in cultural behavior may have been the result of a reassortment and somatic segregation of nuclei of different potentialities that arose through atypical division, or (2) they may have resulted from a fortuitous change in the arrangement and organization of heritable material. The latter interpretation appears to be the more tenable, and until a detailed and comparative cytological study of the variant and normal type cultures is made the possibility of atypical nuclear divisions and subsequent reassortment of these nuclei to account for variation can be advanced only as a hypothesis.

It is not surprising that greater variation in cultural behavior was found in cultures propagated on agar slants than in those carried in Petri dishes. On the limited surface of the agar slant these variations may take place without being observed; in subsequent mass transfers to fresh agar slants a large number of spores and mycelial fragments are carried over, some of which may be of one or more variant cultural types. Continued transferring may well eliminate one or another type until a practically pure culture of a particular and distinct appearance is obtained. Where cultures are carried in Petri dishes and only relatively few spores are isolated from a given colony, the chances of obtaining a variant type culture are considerably reduced even though the variant type may be present but unobservable in the parent colony. If a larger number of conidia had been isolated from colonies carried in Petri dishes, then perhaps greater variation would have been observed.

PATHOGENICITY STUDIES

The pathogenicity studies were undertaken not only with the object of determining the relative virulence of the various cultures, but also with a view to finding a possible correlation between colony type and degree of virulence.

These studies were conducted in benches in the greenhouse where the soil temperature was maintained between 15° and 18° C. The relatively low soil temperature was selected in order to produce a sufficient amount of infection to obtain a differential in virulence between the cultures. Dickson (9)⁵ has already pointed out that seedling blight of corn, caused by *Gibberella sarabini*, is favored by soil temperatures ranging from 8° to 20°, whereas at temperatures above 24° no blighting occurs. No attempt was made to control moisture other than to maintain the soil at a moisture content that would permit normal growth and development of the seedlings.

Two lines of corn, obtained through the department of plant pathology of the University of Wisconsin and produced by J. R. Holbert at Bloomington, Ill., were used in the experiments. One line, designated as R4, has proved for a period of years very susceptible to

⁵ Reference is made by number (italic) to Literature Cited, p. 161.

seedling blight; while under comparable conditions, the other line, Br10, has been relatively resistant. The line R4 was used in all series of the pathogenicity studies, whereas Br10 was used only in series 6 and 7. In all, 45 single isolates, the cultural behavior of which had not been followed so much in detail as had those isolates used in the section called "Cultural Studies", were employed in the first five series of the experiment. Most of these were single ascosporic cultures from individual asci; a few of the cultures, however, were grown from isolated hyphal tips of germinating ascospores. Originally all of these isolates were alike in their cultural behavior; after being propagated for a comparatively short time on potato-dextrose agar, however, certain variations in their appearance became evident. In order to study these variations in greater detail single conidial isolations were made of each isolate where this was possible. In certain variant types spores were no longer produced and hyphal tips were isolated from the mycelium. Descriptive notes and photographs of these cultures later proved useful in making correlations between colony type and virulence.

Inoculations were made by immersing 50-kernel samples of corn in a suspension of spores and mycelial fragments from an individual culture. Since an extreme variation existed in the sporulation of the cultures, ranging from those in which conidia were very abundant to those in which none were produced, standardization of the inoculum was impossible. Notes on the relative abundance of sporulation of each culture were taken to determine whether a correlation existed between the abundance of conidia and virulence. After the corn kernels had been soaked in a suspension of a single culture for 3 to 5 minutes they were planted in the soil in the bench at a depth of 1 inch. Check plantings were immersed in sterile distilled water for the same period of time and planted in the same bench.

When the control seedlings, grown from the uninoculated seed, had reached the third-leaf stage, which required about 30 days at this soil temperature, both the inoculated and uninoculated lots were removed from the soil and classified according to the severity of infection. The classes in which the seedlings were placed were assigned arbitrary values ranging from 10 for healthy seedlings to 0 for those that failed to emerge because of disease. The number of seedlings in each class was multiplied by the given value of that class and the sum of these values for each class was divided by the number of kernels planted. If the quotient is subtracted from 10, the resulting value represents the disease index. Thus, a disease index of 10 indicates that all seedlings had been killed before emergence, whereas a disease index of 0 indicates that all seedlings emerged and were healthy.

The disease indices of the cultures for each of the five series, together with an average disease index for each culture for all series, are given in table 2. It will be seen at once that there were wide differences in pathogenicity in the cultures. In the set of cultures Iowa 211-1 to 8, originally grown from the eight spores, respectively, of a single ascus, such differences are strikingly evident; the average disease indices range from 3.17 to 9.75, showing an extreme difference of 6.58.

TABLE 2.—Disease indices of cultures of *Gibberella saubinetii* used in inoculation studies on an inbred strain of corn, R4

Culture no.	Disease indices for—					Average for—	
	Series 1	Series 2	Series 3	Series 4	Series 5	Individual cultures	Set of cultures
Ill. 111-1	7.76	5.54	4.08	8.20	8.40	6.79	4.66
-2	3.46	2.88	2.68	6.44	5.60	4.21	
-3	8.66	7.92	6.68	9.12	8.52	8.18	
-4	4.00	2.78	1.12	4.92	2.96	3.15	
-5	5.38	4.02	.86	2.58	2.12	2.99	
-6	2.82	1.20	.88	2.44	2.54	1.97	
-7	6.10	4.30	2.84	6.84	6.48	5.31	
Ill. 121-1A	9.60	9.32	8.66	10.00	9.72	9.46	6.55
-1B	6.16	6.50	3.56	7.74	6.40	6.07	
-3A	6.90	7.00	4.44	8.44	7.10	6.77	
-3B	9.62	9.84	8.98	9.96	9.66	9.61	
-6A	5.16	3.48	2.48	6.24	5.68	4.61	
-6B	7.34	6.24	4.18	9.24	8.24	7.05	
-6A	1.40	1.82	.64	1.24	2.96	1.61	
-6B	7.12	5.66	6.58	9.28	7.48	7.22	4.20
Iowa 111-1	.86	1.20	.48	.80	1.24	.91	
-2	6.68	4.98	3.00	6.70	5.36	5.35	
-3	7.40	5.34	3.82	8.88	6.76	6.44	
-4	5.12	3.72	2.72	5.92	5.60	4.61	
-5	2.26	1.40	.72	1.94	1.40	1.54	
-6	.80	.66	.32	1.84	1.00	.92	
-7	8.00	6.34	4.48	8.34	7.72	6.97	7.47
Iowa 211-1	7.10	7.78	3.92	8.94	7.60	7.00	
-2	9.06	9.10	8.60	9.96	8.54	9.05	
-3	6.42	5.06	4.56	7.66	7.08	6.15	
-4	4.88	1.90	2.34	1.86	4.86	3.17	
-5	10.00	9.88	9.24	9.92	9.72	9.75	
-6	5.56	5.22	.66	2.74	2.22	3.30	
-7	9.42	9.62	9.36	9.96	9.74	9.62	3.55
-8	10.00	9.72	9.06	9.90	9.54	9.64	
Minn. 111-1	9.88	8.84	8.30	9.48	8.90	9.07	
-2	4.30	2.36	2.12	5.78	4.52	3.81	
-3	1.00	1.32	.40	1.70	1.12	1.11	
-4	2.64	1.64	.60	2.36	2.60	1.97	
-5	1.04	.88	.32	1.01	1.04	.86	
-6	8.74	8.00	6.48	9.84	9.64	8.54	1.36
-7	1.16	.72	.52	1.40	1.00	.96	
-8	3.76	3.86	1.28	5.82	4.50	3.84	
Minn. 112-4A	7.42	6.76	5.94	7.88	8.58	7.31	
-4B	5.86	2.52	.68	3.92	1.62	2.90	
-6A	2.14	.94	.20	2.40	.64	1.26	
-6B	1.78	1.24	.44	1.46	1.00	1.18	
-7A	.86	1.68	.54	1.04	1.06	1.03	1.36
-7B	.80	.68	.92	1.54	1.62	1.11	
Average indices for all cultures in each series	5.29	4.50	3.36	5.65	5.12	-----	-----
Average for control plantings	.79	.65	.62	1.09	1.37	-----	-----

In every case type-A colonies showing rapid radial growth along with an abundance of aerial mycelium were highly pathogenic. No colonies showing this type of growth were weakly parasitic. A few colonies, however, that did not exhibit such cultural characters were rather virulent. Nevertheless, the indications are that rapid mycelial growth and abundant aerial mycelium are directly correlated with a high degree of pathogenicity in the majority of cases. There may, however, be still other factors responsible for pathogenicity. Similar observations on species of *Fusarium* have been made by other workers. Brown (3), working on certain fruit-rotting species of *Fusaria*, has pointed out that the mycelial type of culture is the most pathogenic. He also states that this type of growth is the form found on first isolations from diseased tissue. Harvey (11), in a study of the parasitic

abilities of cultures of *F. fructigenum* Fr., found that high virulence was correlated with vigorous mycelial growth.

The data obtained on the relative sporulating ability of the cultures indicate that no positive correlation exists between sporulation and pathogenicity. Some of the most virulent cultures, as well as those that show very weak parasitic tendencies, produce conidia in abundance. Likewise, cultures that produce very few spores may or may not be pathogenic. The sporulating ability of the cultures used in the pathogenicity studies was determined by observing the relative number of spores in a drop of inoculum. No actual counts were made.

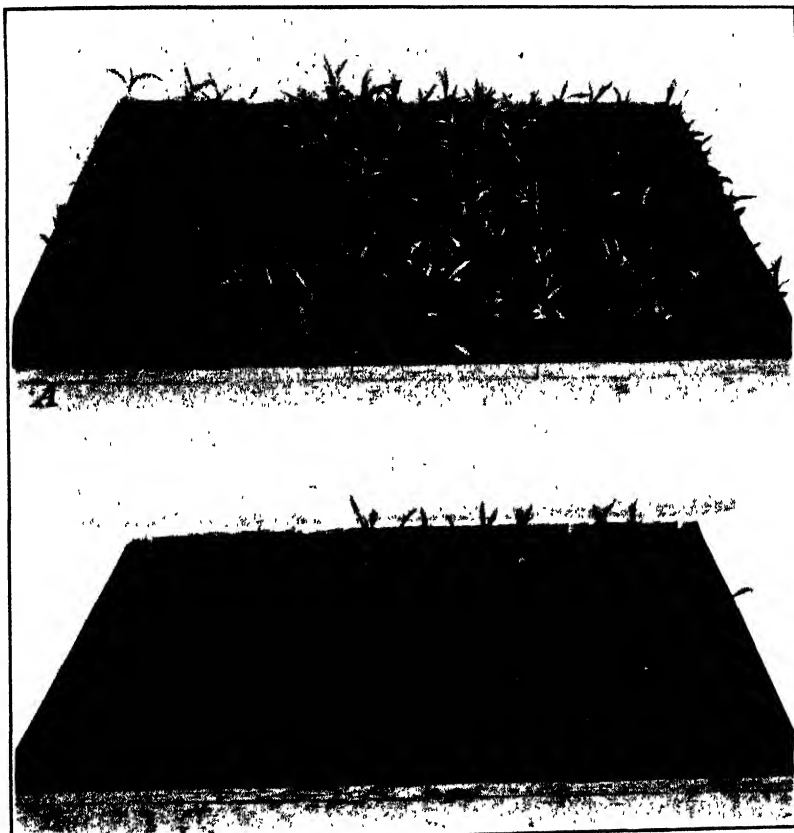


FIGURE 8.—A, Corn seedlings of resistant line, Br10, each row inoculated with a single culture. Note the difference in virulence between the cultures used. B, Corn seedlings of susceptible line, R4, inoculated with the same cultures as in A. Note the relative susceptibility of R4.

In two additional inoculation series (series 6 and 7) both inbred lines of corn, R4 and Br10, were used. In these series an entirely different group of cultures was employed. The same general results were obtained as in the first five series, that is, high virulence was directly correlated with rapid mycelial growth and vigorous aerial mycelium. No indication was found of selective pathogenicity of cultures between two lines of corn, all cultures being more virulent on the susceptible line, R4, than on the resistant line, Br10 (fig. 8 and table 3).

TABLE 3.—*Disease indices of cultures of Gibberella saubinetii used in inoculation studies on 2 inbred lines of corn, Br10 and R4*

Culture no.	Disease indices for indicated culture series on—					
	Inbred Br10			Inbred R4		
	Series 6	Series 7	Average	Series 6	Series 7	Average
Minn. 211-1	8.54	7.62	8.08	9.91	9.72	9.81
-2	8.23	7.80	8.01	9.85	10.00	9.92
-3	8.00	7.58	7.79	9.94	10.00	9.97
-4	7.39	6.58	6.98	9.84	9.68	9.76
-5	8.36	7.94	8.15	9.74	9.86	9.80
-6	8.63	7.66	8.14	9.76	9.20	9.48
-7	7.74	7.90	7.82	9.79	9.84	9.81
-8	4.01	2.94	3.47	8.13	7.80	7.96
Iowa 221-1 (A)	2.09	2.56	2.32	6.57	5.52	6.04
-2	2.53	1.56	2.04	5.57	5.24	5.40
-3	1.95	1.12	1.53	5.40	4.28	4.84
-4	2.19	1.24	1.71	3.99	1.60	2.79
-5	3.73	6.12	4.92	7.35	8.36	7.85
-6	1.18	.64	.91	1.14	.52	.83
-7	8.38	7.12	7.75	9.96	10.00	9.98
-8	5.42	5.28	5.35	9.13	9.32	9.22
Ill. 132-1B-(B)	2.01	1.66	1.78	6.34	5.46	5.90
1B-(B)-S	2.43	1.44	1.93	5.35	3.74	4.54
1B-(A)	8.72	9.20	8.96	9.86	9.76	9.81
1B-(A)-S	4.42	5.46	4.94	8.70	8.88	8.79
Checks (averages)	1.02	.58	.80	1.35	1.19	1.27

EFFECT OF PASSING CULTURES THROUGH THE HOST

Experiments of a preliminary nature were conducted to determine what effect the parasitic habit might have on the cultural behavior of the fungus.

Fifteen lots of corn seedlings, each of which had been inoculated at planting with a different isolate of known cultural behavior, were removed from the greenhouse bench, surface-disinfected, and the diseased tissue plated on hard potato-dextrose agar. After 3 days the fungus had grown out of the diseased portion and could be readily identified as *Gibberella saubinetii*. Mass transfers of apparently pure cultures were made to plates of potato-dextrose agar and 5 days later cultural characters were studied and recorded.

In all cases the reisolated cultures were the same in their cultural behavior as at the time of inoculation, indicating that the sojourn within the host tissue, which was about 1 month, had no perceptible influence on the cultural characters of the fungus. These results are not in accord with the findings of Burkholder (4) in a species of *Fusarium*. He reported morphological and physiological changes attending *F. martii phaseoli* Burk. when kept in culture for a long period of time, but after inoculation and reisolation the fungus assumed its original cultural aspects. He also noted that loss of virulence was restored after two inoculations and reisolations. Coons and Larmer (7) found that aberrant cultures of *Cercospora beticola* Sacc. have a tendency to revert to the original form after inoculation and reisolation from the host.

DISCUSSION

Throughout the present investigations it has been definitely shown that considerable variation exists among single ascosporic cultures and hyphal-tip cultures of the fungus *Gibberella saubinetii* while growing

under an artificial environment. This variability has been expressed both in cultural behavior and pathogenicity.

An extensive volume of literature has been devoted to the subject of variability of fungi in pure culture. Most of the authors, however, simply report the occurrence of the variations as differences in cultural behavior, morphology, physiology, or pathogenicity, without attempting to analyze their true nature.

Several different views regarding the nature of variations in fungi have been put forward by different groups of workers. Stakman (18), Christensen (6), and Stevens (19) believe that most of the variability in fungi probably is due to true mutation or saltation similar to bud mutation in the higher plants. Brown (2) and Mohendra (17) also are of the opinion that variants arise as true mutants. La Rue (14) suggests that loss of virulence in pathogenic fungi is brought about by the appearance of saprophytic strains, which arise as mutants that are able to thrive better under artificial culture and thus outgrow the parasitic strains.

Caldis and Coons (5) have demonstrated that certain variations in the fungi were of a more or less permanent nature and probably induced by nutritional disturbances or a poisoning of the protoplasm. These writers regard such variations as similar to the "Dauermodifikation" described by Jollos (13) for certain Protista.

Holton (12), substantiating some of Dickinson's work (8), has suggested that in some of the smuts, and in other fungi where true sexuality exists, delayed segregation of certain heritable factors may account for the appearance of variants.

Leonian (15, 16) has explained variation in the fungi as a natural phenomenon whereby the culture traces the variability of the species. Attempts by that author to induce mixochimaera in *Fusarium moniliforme* Sheld. were unsuccessful. After two distinct isolates had been grown in combinations for a period, reisolation revealed both parent strains and in addition a new type. The latter was believed to be a dissociant of one of the parent strains and not a heterotype resulting from anastomosis and intimate association of the protoplasm of the two parents.

In opposition to this opinion, Brierley (1) holds that the appearance of variants in many instances may be accounted for by a heterocaryotic condition of the original isolate, and that through subsequent culturing a reassortment of nuclei may take place that gives rise to cultures phenotypically divergent from the parent. Brierley further states that true mutation, defined as "a fundamental change in one or more of the hereditary units, and carried from one generation to another", has not been adequately shown in the fungi. He concedes the possibility of aberrant nuclear divisions by virtue of which new forms might arise. Hanson and Smith (10) have brought out experimental evidence with *Botrytis* in support of Brierley's contention that a reassortment of the nuclei of a heterocaryotic isolate may give rise to strains differing from the original.

In regard to the pathogenicity of different cultures, Tanja (20), working with three isolations of *Gibberella saubinetii* procured in culture from different sources, found that they differed from one another in virulence. Tu (21), in an investigation of species of *Fusarium* causing head blight of small grains, has shown that three isolates of *F. graminearum* Schwabe could be separated on the basis of their viru-

lence on several varieties of wheat and on their growth rates. Some information concerning the changes that went on in the cultures employed by these workers before they were tested for pathogenicity would be of interest.

The nature of the variability observed in the investigations reported here does not appear to be directly due to a condition of heterocaryosis in the original isolates. Theoretically, the four nuclei in an ascospore are of the same genetic constitution, and if segregation occurs it takes place in the ascus before the spores are formed. Likewise, all nuclei in the hypha of a germinating ascospore have their origin in the mother nucleus of the cell from which the hypha was produced, and should be genetically alike. Under these assumptions, barring any aberrant nuclear divisions, duplicate ascosporic cultures from a common isolate and hyphal-tip cultures from the same ascospore should behave in the same manner.

Whether aberrant nuclear divisions occur would be difficult to determine cytologically with the material used in these studies. If atypical divisions of the nuclei take place, it is conceivable that only a few of such aberrances could, through the reassortment and segregation of the nuclear complex of a single culture, give rise to forms differing in several respects from the original cultural type. There is also the possibility, and this is perhaps the most tenable explanation, that variations in cultural behavior and pathogenicity in this organism are brought about by gene changes or chromosomal aberrations. That the variant types have appeared rather suddenly and have in some cases passed unaltered through the ascigerous stage strengthens this supposition.

In the experience of the writer, as well as of some other workers, abnormal cultural types were never found under natural conditions. That these abnormal or variant forms are weak pathogens may preclude their isolation from diseased tissue. In addition, it has been observed that few of these forms pass through the ascigerous stage, which may account for their absence from the isolations made from perithecial material. If these forms do exist in nature they probably do so as saprophytes and are constantly being eliminated through competition and natural selection of the more vigorous forms.

SUMMARY

Single ascosporic and hyphal-tip cultures of *Gibberella saubinetii* (Mont.) Sacc. were studied in relation to cultural behavior and pathogenicity.

The method employed in isolating the eight ascospores from a single ascus is described in detail.

All original ascosporic and hyphal-tip isolates were strikingly similar in cultural behavior regardless of the locality from which the perithecial material was collected. During the course of the studies considerable variability was observed in cultural behavior. The variation was more or less haphazard and did not appear as a result of an orderly segregation within the ascus.

Wide differences were found among the isolates with respect to their ability to cause seedling blight on corn. Some isolates were highly virulent while others were practically nonpathogenic.

A direct correlation was found between colony type and virulence. Those cultures that showed a rapid radial growth and an abundance

of aerial mycelium were always highly pathogenic, whereas those having a relatively slow growth rate and a pionnotes type of growth were generally poor pathogenes. No correlation could be established between abundant conidial production and degree of pathogenicity.

Passage through the host apparently had no influence on the cultural characters of the isolates.

It is suggested that the variability observed in the present investigations may be due to 1 of 2 possible causes: (1) Abnormal nuclear divisions with subsequent reassortment and segregation of a new nuclear complex, or (2) the existence of true mutants.

LITERATURE CITED

- (1) BRIERLEY, W. B.
1922. DISCUSSION ON MUTATION OF SPECIES. Brit. Med. Jour. 2: 722-726, illus.
- (2) BROWN, W.
1926. STUDIES IN THE GENUS FUSARIUM. IV. ON THE OCCURRENCE OF SALTATIONS. Ann. Bot. [London] 40: [223] 243, illus.
- (3) ———
1928. STUDIES IN THE GENUS FUSARIUM. VI. GENERAL DESCRIPTION OF STRAINS, TOGETHER WITH A DISCUSSION OF THE PRINCIPLES AT PRESENT ADOPTED IN THE CLASSIFICATION OF FUSARIUM. Ann. Bot. [London] 42: [285] 304.
- (4) BURKHOLDER, W. N.
1925. VARIATIONS IN A MEMBER OF THE GENUS FUSARIUM GROWING IN CULTURE FOR A PERIOD OF FIVE YEARS. Amer. Jour. Bot. 12: 245-253.
- (5) CALDIS, P. D., and COONS, G. H.
1926. ACHROMATIC VARIATIONS IN PATHOGENIC FUNGI. Mich. Acad. Sci., Arts, and Letters, Papers 6: 189-236, illus.
- (6) CHRISTENSEN, J. J.
1925. PHYSIOLOGIC SPECIALIZATION AND MUTATION IN HELMINTHOSPORIUM SATIVUM. Phytopathology 15: [785]-795, illus.
- (7) COONS, G. H., and LARMER, F. G.
1930. THE PHYSIOLOGY AND VARIATIONS OF CERCOSPORA BETICOLA IN PURE CULTURE. Mich. Acad. Sci., Arts, and Letters, Papers 11: 75-104, illus.
- (8) DICKINSON, S.
1931. EXPERIMENTS ON THE PHYSIOLOGY AND GENETICS OF THE SMUT FUNGI CULTURAL CHARACTERS. PART II.—THE EFFECT OF CERTAIN EXTERNAL CONDITIONS ON THEIR SEGREGATION. Roy. Soc. [London] Proc., Ser. B, 108: 395-423.
- (9) DICKSON, J. G.
1923. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON THE DEVELOPMENT OF SEEDLING-BLIGHT OF WHEAT AND CORN CAUSED BY GIBBERELLA SAUBINETII. Jour. Agr. Research 23: 837-870, illus.
- (10) HANSON, H. N., and SMITH, R. E.
1932. THE MECHANISM OF VARIATION IN IMPERFECT FUNGI: BOTRYTIS CINEREA. Phytopathology 22: 953-964, illus.
- (11) HARVEY, C. O.
1929. STUDIES IN THE GENUS FUSARIUM. VII. ON THE DIFFERENT DEGREES OF PARASITIC ABILITY SHOWN BY VARIOUS STRAINS OF FUSARIUM FRUCTIGENUM. Ann. Bot. [London] 43: [245]-259.
- (12) HOLTON, C. S.
1932. STUDIES IN THE GENETICS AND CYTOLOGY OF USTILAGO AVENAE AND USTILAGO LEVIS. Minn. Agr. Expt. Sta. Tech. Bull. 87, 34 pp., illus.
- (13) JOLLOS, V.
1920. EXPERIMENTELLE VERERBUNGSTUDIEN AN INFUSORIEN. Ztschr. Induktive Abstam. u. Vererbungslehre 24: [77]-97, illus.
- (14) LA RUE, C. D.
1925. LOSS OF VIRULENCE IN FUNGI. Science (n. s.) 62: 205-206.

- (15) LEONIAN, L. H.
1930. ATTEMPTS TO INDUCE "MIXOCHIMAERA" IN *FUSARIUM MONILIFORME*. *Phytopathology* 20: 895-901, illus.
- (16) ———
1932. THE PATHOGENICITY AND VARIABILITY OF *FUSARIUM MONILIFORME* FROM CORN. *West Va. Agr. Expt. Sta. Bull.* 248, 15 pp., illus.
- (17) MOHENDRA, K. R.
1928. A STUDY IN THE CHANGES UNDERGONE BY CERTAIN FUNGI IN ARTIFICIAL CULTURE. *Ann. Bot. [London]* 42: [863]-887, illus.
- (18) STAKMAN, E. C., CHRISTENSEN, J. J., EIDE, C. J., and PETURSON, B.
1929. MUTATION AND HYBRIDIZATION IN *USTILAGO ZEAE*. . . *Minn. Agr. Expt. Sta. Tech. Bull.* 65, 108 pp., illus.
- (19) STEVENS, F. L.
1922. THE *HELMINTHOSPORIUM* FOOT-ROT OF WHEAT, WITH OBSERVATIONS ON THE MORPHOLOGY OF *HELMINTHOSPORIUM* AND ON THE OCCURRENCE OF SALTATION IN THE GENUS. *Ill. State Nat. Hist. Survey Bull.* 14: [77]-185, illus.
- (20) TANJA, A. E.
1933. UNTERSUCHUNGEN ÜBER *GIBBERELLA SAUBINETII* (DUR. ET MONT.) SACC. UND DIE *FUSARIOSE* DES WEIZENS. *Phytopath. Ztschr.* 6: 375-428, illus.
- (21) TU, C.
1929. PHYSIOLOGIC SPECIALIZATION IN *FUSARIUM* SPP. CAUSING HEAD-BLIGHT OF SMALL GRAINS. *Phytopathology* 19: 143-154, illus.

RATES OF GROWTH AND NITROGEN ASSIMILATION OF HAVANA SEED TOBACCO¹

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INTRODUCTION

This investigation was planned to furnish basic information for the intelligent study of fertilizer practices, with particular reference to quantity, source, and time of application of nitrogenous materials. It involved measurements of rates of growth and nitrogen intake of the Havana Seed tobacco crop in the Connecticut Valley during the 5-year period 1929-33. The various plant parts (roots, stalk, crop leaves, sucker leaves, and seed pods) were differentiated in the analyses; hence, a picture of the increase in dry weight and total nitrogen content of this crop is presented by the data.

No effort has been made to interpret these data in relation to the various theories concerning the mathematical laws underlying growth rates, but it is hoped that those interested will avail themselves of this material to test the validity of these theories.

PREVIOUS INVESTIGATIONS

Kosutány² presented data on the total dry weight and chemical analysis of tobacco at six dates, from the transplanted seedlings on May 31 to the time of ripening of the seeds on September 17. For comparison with data in the present study, his results, calculated in pounds per acre on the basis of an equal number of plants per acre, are shown in table 1. As to whether the total weight of the plants included roots was not stated.

TABLE 1.—*Total dry weight and nitrogen content of tobacco during its development (after Kosutány)*

Age of plant in field (days)	Total dry weight (pounds per acre)	Total nitrogen (pounds per acre)	Nitrogen	Age of plant in field (days)	Total dry weight (pounds per acre)	Total nitrogen (pounds per acre)	Nitrogen
			Percent				Percent
0.....	10.8	0.20	1.85	55.....	1,230.0	36.60	2.98
21.....	81.0	2.20	2.72	77.....	4,708.0	110.60	2.35
43.....	643.6	22.60	3.51	99.....	4,100.0	94.20	2.30

Davidson³ in data based on the average of three tobacco varieties at three stages of growth (plant bed, topping, and cutting) shows average nitrogen contents of 2.88, 2.97, and 2.73 percent, respectively, at the successive stages, for the whole plant. The leaf at the topping stage contained 4.40 percent of nitrogen, as compared with 3.66 percent at cutting time.

¹ Received for publication Mar. 16, 1935; issued September, 1935.

² KOSUTÁNY, T. CHEMISCH-PHYSIOLOGISCHE UNTERSUCHUNG CHARACTERISTISCHERER TORAKSORTEN UNGARNS. p. 41. Budapest. 1882.

³ DAVIDSON, R. J. ANALYSES OF PARTS OF TORACCO PLANT AT DIFFERENT STAGES OF GROWTH. Va. Agr. Expt. Sta. Bull. 50 (n. s. v. 4, no. 3), pp. 35-52. 1895.

Garner and his coworkers⁴ measured the growth of tobacco at successive stages on the basis of height of plant to lowermost leafless branch when different forms and amounts of nitrogen were applied in the fertilizer. Sigmoid growth curves, with maximum rate of growth between August 11 and August 26, were obtained with application of no nitrogen and with 40 pounds of nitrogen applied as nitrate of soda. With 20 and 80 pounds of nitrogen applied as nitrate of soda, and 40 pounds as ammonium nitrate, all plants showed no significant reduction in rate of height growth during the final period (Aug. 26 to Sept. 4). The plants were not topped in this experiment.

Vickery, Pucher, Wakeman, and Leavenworth⁵ have made studies of rate of growth and nitrogen assimilation of shade-grown tobacco in Connecticut based on results during one season. Their results include measurements of various forms of nitrogen in the plant. Roots were not included. Rates of dry-matter production and nitrogen intake follow trends in close agreement with the data here presented.

Miller⁶ gives a comprehensive review of the literature dealing with theories in regard to rates of plant growth.

PROCEDURE

The plants measured in this experiment were selected from a plot of tobacco located on typical Connecticut Valley tobacco soil (Merrimac sandy loam.) Previous to setting, heavy applications of fertilizer, containing 200 pounds of nitrogen (four-fifths as organic nitrogen, chiefly cottonseed meal; one-fifth as nitrate of soda), 100 pounds of phosphoric acid, and 200 pounds of potash, were made. Plants were collected at approximately 10-day intervals. Plants of the same apparent size as those withdrawn were tagged for identification to be measured at the subsequent period. In all cases the plants measured were chosen as a fair average of the plot.

The entire plant was removed, including all obtainable roots. The parts were separated and dried immediately in an oven. The number used for measurement ranged from 20 or more at time of setting to 1 after mid-season, in order to furnish a sufficient amount of material for analysis.

After oven-drying, the various portions of the plant were weighed, ground, and analyzed for total nitrogen by the Kjeldahl method.

At the time of topping for the tobacco on the plot, a sufficient number of plants were left untopped to provide samples of untopped plants up to the time of maturity of the seed pods, in comparison with those normally topped.

⁴ GARNER, W. W., BACON, C. W., BOWLING, J. D., and BROWN, D. E. THE NITROGEN NUTRITION OF TOBACCO. U. S. Dept. Agr. Tech. Bull. 414, 78 pp., illus. 1934.

⁵ VICKERY, H. B., PUCHER, G. W., WAKEMAN, A. J., and LEAVENWORTH, C. S. CHEMICAL INVESTIGATIONS OF THE TOBACCO PLANT V. CHEMICAL CHANGES THAT OCCUR DURING GROWTH. Conn. State Agr. Expt. Sta. Bul. 376. 1935.

⁶ MILLER, E. C. PLANT PHYSIOLOGY, WITH REFERENCE TO THE GREEN PLANT. pp. 802-806. New York and London. 1931.

DATA AND DISCUSSION OF RESULTS

For the sake of brevity, the data for the individual years of the experiment (1929, 1930, 1931, 1932, 1933) are not presented. In all cases a similar growth curve was obtained. There was a considerable divergence in the time that elapsed before the beginning of the grand period of growth. In 1929 and 1933, maximum growth rate began at the thirty-fifth day; in 1930, at the thirtieth day; in 1931, at the forty-fifth day; and in 1932, at the fiftieth day. The yields in the two latter years were abnormally low, while in 1930 the greatest growth was attained. In 1929 the crop was destroyed by hail at the sixtieth day, having then attained a growth similar to 1933. In order to weight the data properly, the 1929 crop was assumed to have continued to follow the trend of the 1933 crop for the remaining time.

The dry weights of the various plant parts, based on averages for the 5 years, are given in table 2.

These data are shown graphically in figure 1 by means of smoothed curves. A differentiation of crop and sucker leaves on the topped plants was approximated, on the basis of unpublished data by Berthold⁷ of studies made at Windsor in 1930.

TABLE 2.—*Dry weight and nitrogen content of, and total quantity of nitrogen in, various portions and the entire plant of topped and untopped Havana Seed tobacco, and also in the entire crop, at different times during the growth period, 1929-33*

[The data are averaged for a 5-year period and the mean age of harvest was 72 days]

DRY WEIGHT

Age of plants in field (days)	Roots	Stalks	Leaves	Pods	Total	Probable error of mean ¹
	<i>Pounds per acre</i>	<i>Pounds per acre</i>	<i>Pounds per acre</i>	<i>Pounds per acre</i>	<i>Pounds per acre</i>	
10	0.99	10.96	10.05	12.00	32.00	±0.76
20	2.23	4.38	30.50	37.11	74.22	±4.34
30	12.73	31.35	145.92	190.00	480.00	±27.50
40	82.71	189.05	572.24	844.00	1,488.00	±143.00
50	218.00	502.00	1,260.00	1,980.00	4,000.00	±219.00
60:						
Topped ²	400.00	1,059.00	1,766.00	3,285.00	6,510.00	±83.00
Untopped	475.00	1,375.00	1,820.00	130.0	3,800.00	±172.00
70:						
Topped ²	850.00	1,592.00	2,100.00	4,542.00	9,084.00	±251.00
Untopped	900.00	2,385.00	2,190.00	400.0	5,875.00	±295.00
80:						
Topped ²	1,444.00	1,947.00	2,340.00	5,730.00	11,461.00	±196.00
Untopped	1,475.00	3,040.00	2,490.00	775.0	7,780.00	±313.00

TOTAL QUANTITY OF NITROGEN

10	0.03	0.03	0.37	0.43	0.04
20	.06	.15	1.13	1.34	.37
30	.35	.98	5.61	6.94	1.37
40	2.19	5.37	22.55	30.11	6.48
50	4.86	12.40	49.14	66.40	7.95
60:					
Topped ²	8.97	22.03	64.99	95.99	4.96
Untopped	9.03	28.46	68.25	6.50	7.06
70:					
Topped ²	14.45	28.62	69.30	112.37	7.54
Untopped	14.22	40.55	73.80	16.00	8.31
80:					
Topped ²	19.19	31.74	69.03	119.96	6.88
Untopped	17.26	40.43	69.72	27.59	11.97

¹ From formula $P. E. M. = 0.6745 \sqrt{\Sigma d^2 / n(n-1)}$.

² Topped at 54 days (mean date).

³ Estimated graphically.

⁷ BERTHOLD, K. T. NITROGEN ASSIMILATION—TOPPING AND SUCKERING EXPERIMENT. Unpublished manuscript on file at Tobacco Substation, Conn. State Agr. Expt. Sta. 1931.

TABLE 2.—Dry weight and nitrogen content of, and total quantity of nitrogen in, various portions and the entire plant of topped and untopped Havana seed tobacco, and also in the entire crop, at different times during the growth period, 1929–33.—Continued

[The data are averaged for a 5-year period and the mean age of harvest was 72 days]

NITROGEN CONTENT, PERCENT

Age of plants in field (days)	Roots	Stalks	Leaves	Pods	Total	Probable error of mean
	Pounds per acre	Pounds per acre	Pounds per acre	Pounds per acre	Pounds per acre	
10.....	2.75	3.52	3.65	-----	3.56	0.31
20.....	2.78	3.36	3.70	-----	3.61	.36
30.....	2.77	3.13	3.85	-----	3.65	.24
40.....	2.65	2.84	3.94	-----	3.57	.32
50.....	2.23	2.47	3.90	-----	3.37	.20
60.....						
Topped ¹	1.95	2.08	3.68	-----	2.92	.18
Untopped.....	1.90	2.07	3.75	5.00	2.95	.17
70.....						
Topped ¹	1.70	1.80	3.30	-----	2.47	.16
Untopped.....	1.58	1.70	3.37	4.15	2.47	.14
80.....						
Topped ¹	1.33	1.63	2.95	-----	2.10	.23
Untopped.....	1.17	1.33	2.80	3.56	1.99	.21

¹ Topped at 54 days (mean date).

³ Estimated graphically.

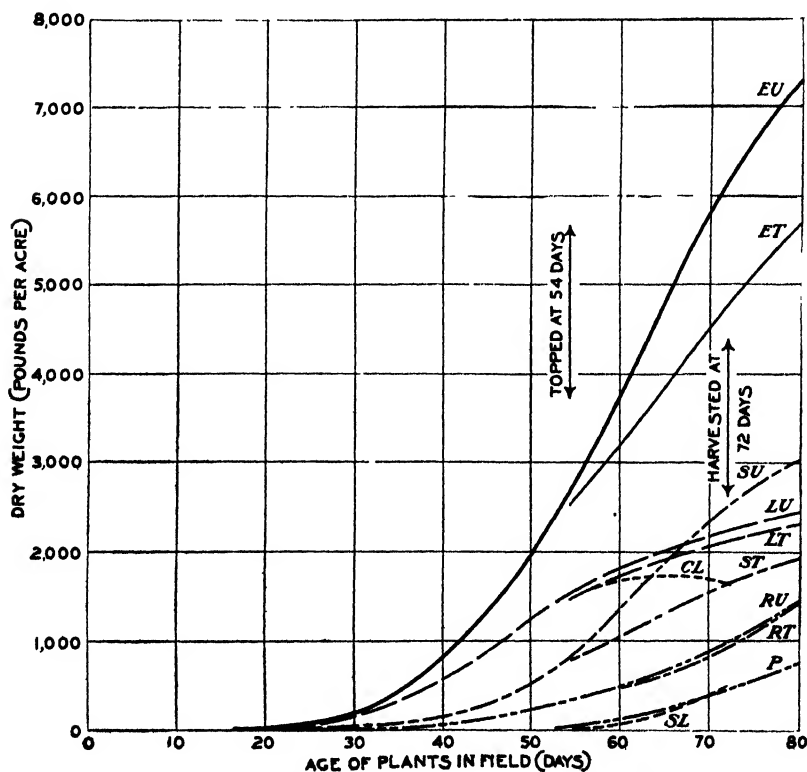


FIGURE 1.—Growth curves of various portions of topped and untopped Havana seed tobacco: EU, entire plant, untopped; ET, entire plant, topped; LU, leaves, untopped plants; LT, leaves, topped plants; CL, crop leaves, topped plants; SL, sucker leaves, topped plants; SU, stalks, untopped plants; ST, stalks, topped plants; RU, roots, untopped plants; RT, roots, topped plants; P, pods, untopped plants.

GROWTH OF NORMAL UNTOPPED PLANTS

As may be seen from an examination of the charts, the entire plant showed a logarithmic acceleration of growth rate, reaching a maximum between the fortieth and sixtieth days after setting in the field. There was only a slight reduction in total growth with approaching seed-pod maturity. The leaf production was diminished after the fifty-fifth day, at about the time the floral parts emerge. The stalk continued rapid growth up to about the sixty-fifth day, its grand period of growth being about 10 days later than for the leaves. The roots apparently attained their maximum rate of growth about when the measurements were ended, on the average at the eightieth day.

CHANGES IN GROWTH AFTER TOPPING

Removal of the terminal bud and a small section of the end of the stalk along with it had a marked effect on the subsequent course of growth of the various plant parts. The total dry-matter production was markedly diminished, out of all proportion to the amount of dry matter removed in the topping operation (estimated at about 100 pounds per acre). The major difference is due to the decreased stalk growth of the topped plant. There was comparatively little difference in total leaf production, and root growth was nearly identical. No pods were produced on the topped plant, this accounting for some of the difference in total dry matter.

However, it is to be noted that the crop leaves, all being developed at the time of topping, grew very little after topping, and apparently suffered some loss in weight just prior to the harvest date. At the same time the sucker leaves were growing rapidly. Crop and sucker leaves are not differentiated in the final data for the eightieth day, nor for the untopped plant at any time.

NITROGEN CONTENT OF PLANTS

The average nitrogen content, on a percentage basis, for the various parts of the plants at the different dates, is shown in table 2, and graphically in figure 2.

Some interesting relationships are evident. Stalks and leaves, both contained approximately 3.5 percent nitrogen at first sampling and then rapidly diverged. The stalks decreased progressively in nitrogen content to a minimum of about 1.5 percent at the final period, although those of the topped plants apparently showed some decline in rate of decrease, possibly due to restoration of vegetative conditions.

The decreased nitrogen content of the stalk is doubtless associated with the development of woody tissue, so prominent in mature tobacco stalks.

The leaves gradually increased to a maximum of about 3.95 percent nitrogen at about 40 days, or early in the grand period of growth of the entire plant. The percentage content of nitrogen of the combined crop and sucker leaves from the topped plants was at first lower than the untopped. Later this condition was reversed, due to the production of sucker leaves of high nitrogen content. The crop leaves decreased in nitrogen content at a very rapid rate after topping, on the basis of Berthold's data.⁸

⁸ Berthold, K. T. See footnote 7.

The roots showed a slight increase in nitrogen content up to the thirtieth day, with subsequent progressive decrease. The topping operation apparently tends to produce a temporary storage of nitrogen in the roots, since the normal rate of nitrogen decrease is slowed down for about 10 days. It is to be noted that the maximum nitrogen content in the roots occurred before that in the leaves.

Pods, when first produced in the bud stage, had a very high nitrogen content of over 5 percent, which decreased rapidly during their

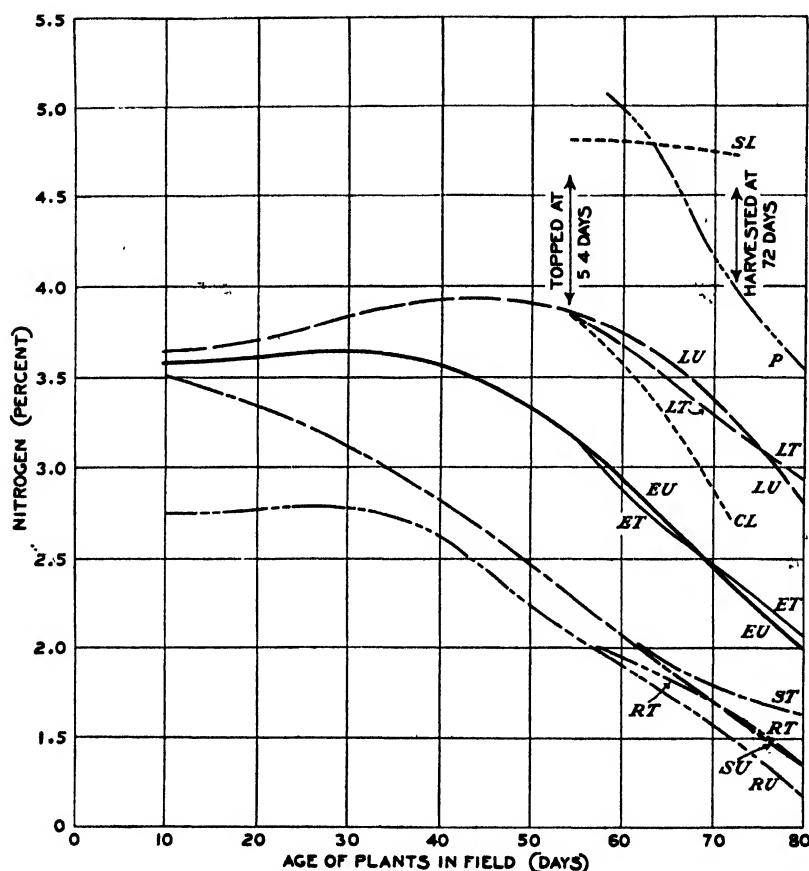


FIGURE 2- Nitrogen content of various portions of topped and untopped Havana seed tobacco: EU, Entire plant, untopped; ET, entire plant, topped; LU, leaves, untopped plants; LT, leaves, topped plants; CL, crop leaves, topped plants; SL, sucker leaves, topped plants; ST, stalks, topped plants; RT, roots, topped plants; RU, roots, untopped plants; P, pods, untopped plants.

development. The measurement was approximately the same as for the seed itself, the latter making up most of the entire weight of the pods.

TOTAL NITROGEN INTAKE OF THE PLANTS

The final objective of this experiment was the measurement of total nitrogen intake by the tobacco plant at various periods of growth. This is the resultant of the two previous sets of measurements and is shown in table 2 and graphically in figure 3.

While on the whole the same general picture is presented as for total dry weight, it is seen that the decline in the rate of nitrogen intake was more rapid during the later stages, due to the previously noted decreases in nitrogen content of the various parts of the plant. Thus a sigmoid curve for total nitrogen in the entire plant is more clearly in evidence, especially for the topped plants.

The translocation of nitrogen from the leaves of the untopped plant and from the crop leaves of the topped plants, is clearly shown. The stalks, despite their continued increase in total weight during the final period, had practically ceased to be a factor in building up the nitrogen in the plant.

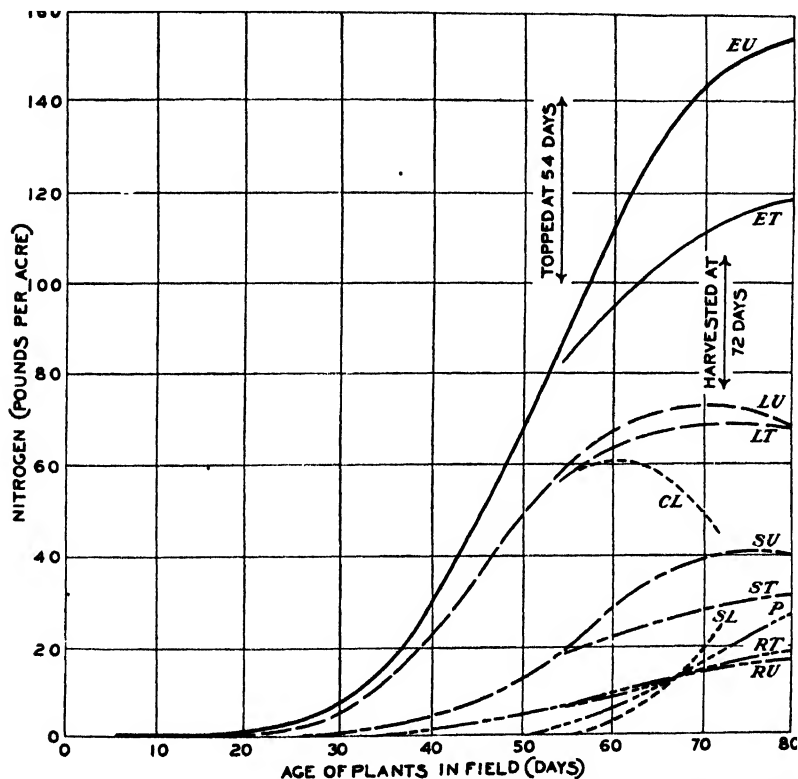


FIGURE 3.—Amounts of nitrogen contained in various portions of topped and untopped Havana seed tobacco in the course of the growth period: EU, Entire plant, untopped; ET, entire plant, topped; LU, leaves, untopped plants; LT, leaves, topped plants; CL, crop leaves, topped plants; SL, sucker leaves, topped plants; SU, stalks, untopped plants; ST, stalks, topped plants; P, pods, untopped plants; RT, roots, topped plants; RU, roots, untopped plants.

Although growth was still active at the final measurement, nitrogen intake from the soil was rapidly approaching cessation. The building up of nitrogen in the pods of the untopped plant occurred largely at the expense of its leaves.

Under the scheme of fertilization practiced on the field, with 80 percent of the nitrogen applied in the organic form, the amount of nitrate nitrogen in the soil attained a maximum of about 60 pounds per acre in about 3 weeks after setting. Except as interrupted by

leaching from the soil by unusually heavy rains, the nitrate nitrogen liberated by biological activity kept pace with crop removal until about the first of August, normally about 60 days after setting. The nitrates in the soil then decreased rapidly, falling off to less than 10 pounds per acre at the time of final measurement in this experiment. These observations are based on data as to nitrate nitrogen in the soil on the plots from which the growth samples were taken during 1932 and 1933, and in the soils of plots under various nitrogenous fertilizers during 1932, 1933, and 1934.

It is evident that, since the nitrate level of the soil was high until the sixtieth day, there was no limitation in nitrogen intake until this time. It is of interest to note that the untopped plants showed a definite diminution in rate of total nitrogen intake during the time of depletion of nitrates in the soil.

Table 3 gives a picture of the distribution of dry weight and nitrogen in the Havana seed tobacco crop at harvest date. These data were obtained by interpolation from the graphs based on average results over the 5-year period.

TABLE 3.—*Dry weight and nitrogen content of various parts of the Havana seed tobacco crop at harvest date, 72 days after setting*

[Average data, 1929-33]

Plant part	Dry weight	Nitrogen		Nitrogen contained in entire plant
	Pounds	Percent	Pounds	Percent
Roots.....	950	1.58	15.0	13.2
Stalks.....	1,675	1.77	29.6	26.0
Suckers.....	507	4.85	24.6	21.5
Crop leaves.....	1,643	2.73	44.9	39.3
Total.....	4,775	2.39	114.0	

PRACTICAL APPLICATION

On the basis of these results an ideal system of nitrogenous fertilization would provide during the first month only moderate amounts, not to exceed 20 pounds, of nitrate nitrogen per acre. Any larger amount would not be required by the crop, and would be subject to loss from the soil by leaching as a result of heavy rains. Thereafter rapidly increasing amounts of nitrates should be supplied, to a total of about 120 pounds per acre at the end of the second month. If this amount is materially exceeded, luxury consumption of nitrogen in the final stages of growth may interfere with the normal decline of the nitrogen content in the crop leaves. The tobacco fertilization normally practiced in the Connecticut Valley substantially accomplishes these ends, although nitrates are provided in somewhat excessive amounts during the early growth period. Usually 40 pounds of nitrate nitrogen are applied with the fertilizer, and available nitrogen from organic sources builds up the soil above this level until the beginning of rapid nitrogen intake by the crop. However, nitrogen supply for the season as a whole is apparently well adjusted to the demands of the crop. Although 160 pounds per acre of organic nitrogen are

provided, only about 60 percent of this amount is available during the season, on the basis of data from lysimeter investigations at Windsor.⁹

A duplication of these conditions when using inorganic nitrogen sources is difficult under Connecticut Valley conditions. The application of 120 pounds of nitrogen in such materials at planting time may result in an inadequate supply for normal yield if there is serious leaching from the soil, while, in a season without leaching, larger amounts may prove excessive. Inorganic nitrogen applications may be used in fractional doses, proportional to the demands of the crop during the subsequent period, but in case the soil is dry when these applications are made, they may not diffuse to within reach of the root system in time to be used when they are needed.

It is believed that these data in part explain the general failure of attempts to use large proportions of nitrates or other inorganic sources of nitrogen for the tobacco crop in the Connecticut Valley. They provide a working basis for more intelligent future attempts along this line.

SUMMARY

The rate of growth and of nitrogen intake by the Havana seed tobacco crop was measured for the five seasons, 1929-33. Separate data are presented for the various parts of the plant, including roots, stalks, leaves, and seed pods.

The early growth for the first 30 days after setting was slow, and was followed by a rapidly accelerating increase. Between the thirty-fifth and fifty-fifth day after setting, the crop attained approximately 50 percent of the dry weight attained at harvest time, and extracted from the soil about 60 percent of its total nitrogen requirement.

Topping interrupted normal development, to the extent that the topped plants, even when permitted to grow beyond the normal harvest date to the time of practical maturity of seeds on the untopped plants, failed by more than 1,500 pounds to attain as great a production of dry matter. This difference is primarily due to decreased stalk growth and the prevention of normal terminal seed-pod production. The increased production of sucker leaves on the topped plant was not in excess of the additional production of normal leaves on the untopped plant.

The nitrogen content of leaves attained a maximum about 40 days after setting. The stalk became decreasingly lower in nitrogen content throughout the growth of the crop. The roots increased slightly in nitrogen content for about 30 days, after which they became progressively poorer in nitrogen. The crop leaves lost nitrogen after the plant was topped.

At the time of cutting, tobacco had extracted 114 pounds of nitrogen from the soil, of which 39.3 percent was contained in the crop leaves, 21.5 percent in the sucker leaves, 26 percent in the stalk, and 13.2 percent in the roots.

In order to meet the nitrogen requirements of the crop at various stages of its growth, without providing amounts so greatly in excess of immediate requirements as to be lost from the soil by leaching, a fertilizer should supply not to exceed one-fifth of its nitrogen in the

⁹ MORGAN, M. F., STREET, O. E., and JACOBSON, H. G. M. FERTILIZER LOSSES THROUGH LEACHING AS MEASURED BY LYSIMETER EXPERIMENTS. Conn. State Agr. Expt. Sta. Bull. 326: 432-441. 1931.

nitrate form during the first month, and a total of approximately 120 pounds of available nitrogen within 2 months after setting. If the latter amount is greatly exceeded, too much nitrogen may be taken up by the crop during the period immediately prior to cutting, thus causing poor quality due to excessive nitrogen content in the crop leaves. Such a condition is being provided by the present practice of applying 40 pounds of nitrate nitrogen and 160 pounds of organic nitrogen just prior to setting, although this results in a nitrate level in the soil much in excess of immediate requirements for at least a month, and serious losses may occur when heavy rains come during this period. If larger amounts of inorganic nitrogen are to be used in the Connecticut Valley tobacco crop, they should be adjusted to the periodic needs for this element.

THE DIGESTIBLE NUTRIENTS OF NAPIER GRASS AND CROTALARIA INTERMEDIA SILAGES, NATAL GRASS HAY, AND THE DRIED REFUSES OF GRAPEFRUIT AND ORANGE CANNERIES¹

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INTRODUCTION

In Florida, where tropical and subtropical forage crops are grown, the summer rainy season makes it difficult to cure hay, and consequently increasing amounts of forage are being conserved as silage. Although standard feeds in other parts of the United States have been investigated thoroughly, little appears to be known of the composition and digestibility of a considerable number of forage crops and byproducts now in use in the southeastern part of the country. One type of feed, new in this section, is the dried refuse from the citrus canning plants. There is promise that this byproduct may be an important carbohydrate feed.

This paper presents the results of a study to determine the composition, coefficients of digestibility, and digestible nutrients of silages made from Napier grass (*Pennisetum purpureum* Schum.) and *Crotalaria intermedia* Kotschy, of hay from Natal grass (*Tricholaena rosea* Nees), and of dried refuse from grapefruit and orange canneries.

METHODS

The methods used in conducting the digestion trials were essentially those recommended by Forbes and Grindley.³ The basal ration consisted of prime cottonseed meal (41 percent total crude protein) and No. 1 Federal grade alfalfa hay. In the experimental rations, one-half of the alfalfa hay was replaced by an equal quantity of the experimental feed, except in the case of silages, where the rate was 3 pounds of silage to 1 pound of hay. In every case, the total ration provided an excess of digestible crude protein above the calculated requirements.

Preliminary feeding periods were 10 days in length, and the experimental periods were from 15 to 20 days. The trials with Napier grass silage totaled 45 "steer-days," and all other trials 80 steer-days each.

¹ Received for publication Apr. 13, 1935; issued September 1935.

² The data dealing with *Crotalaria intermedia* were obtained from a cooperative investigation of the feeding value of crotalarias conducted jointly by the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Departments of Agronomy and Animal Husbandry of the Florida Agricultural Experiment Station. The digestion trials were conducted by the Department of Animal Husbandry. The silage used was made from forage provided by the Division of Forage Crops and Diseases and the Department of Agronomy. G. E. Ritchey was in charge locally for the Division of Forage Crops and Diseases.

³ FORBES, E. B., and GRINDLEY, H. S. ON THE FORMULATION OF METHODS OF EXPERIMENTATION IN ANIMAL PRODUCTION. Bull. Natl. Research Council 6, pt. 2, no 33: 17-27. 1923.

Analyses were made by the methods of the Association of Official Agricultural Chemists.⁴ Determinations of nitrogen were made in triplicate on the fresh feces each day in order to avoid losses of nitrogen. All other constituents were determined on 5-day composite samples.

The coefficients of digestibility were obtained by indirect calculation, taking into account the data from the trials with the basal ration for alfalfa hay, and the average coefficients of digestibility given by Henry and Morrison⁵ for the grade of cottonseed meal used.

PRESENTATION AND DISCUSSION OF RESULTS

No difficulty was experienced in getting the animals to eat the experimental rations. In general, satisfactory agreement among the results for the different animals and for successive 5-day periods was secured, except in the trials with Napier grass silage. The results for 1 of the 4 animals were discarded in this instance, the other 3 being consistent. The greatest discrepancies appeared in those instances where the intake of the particular feed constituent from the experimental feed was but a small fraction of the total intake of that constituent. However, any error from this source introduced into the calculation of the total digestible nutrient content of a feed is relatively small.

The composition, coefficients of digestibility, and digestible nutrient content of the experimental feeds, together with similar standard feeds for purposes of comparison, are presented in table 1.

The silage from Napier grass, harvested as the heads began to appear, was low in crude protein and high in crude fiber. A high fiber content is presumed to depress the digestibility of forages. This appears to have been the case in this instance, as is seen when the nutrient content of Napier grass silage is compared with that of corn silage, and is most apparent when the nutrients are computed on the dry-matter basis.

Harvest of the *Crotalaria intermedia* was delayed in 1933 by heavy rains, so that the crop became more fibrous than is desirable. The high fiber content of this plant at such a stage of maturity appears to have depressed the digestibility of the nutrients, as is seen in comparisons with soybean silage and alfalfa hay. In common with other legume silages, that of *C. intermedia* contained a greater proportion of crude protein in the dry matter than is present in grasses at a similar stage of maturity.

Even though the harvest of Natal grass must wait until the close of the rainy season, and its growth habits tend toward a higher fiber content, its total digestible nutrients were only slightly below those of timothy hay.

Dried grapefruit and orange cannery refuses were both very palatable to cattle. They had a laxative effect when fed in such quantities that they supplied approximately one-half of the dry matter of the ration. Both of these citrus byproducts were low in protein,

⁴ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Compiled by the committee on editing methods of analysis. Revised to July 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.

⁵ HENRY, W. A., and MORRISON, F. B. FEEDS AND FEEDING; A HANDBOOK FOR THE STUDENT AND STOCKMAN. Ed. 18, pp 709-743, illus. Madison, Wis. 1923.

crude fiber, and fat, but high in digestible nitrogen-free extract. The digestion trials place these feeds in the class of high carbohydrate concentrates.

TABLE 1.—The percentage composition, coefficients of digestibility, and digestible nutrients of the experimental feeds, as compared with those of similar standard feeds

Kind of feed	Number of analyses or trials	Dry matter	Crude protein	Crude fiber	Nitrogen-free extract	Crude fat	Ash	Total digestible nutrients	On dry-matter basis	
									Crude protein	Total digestible nutrients
Napier grass silage	3	32.54	1.17	14.45	14.42	0.68	1.82			
Corn silage, well matured ¹	121	26.30	2.10	6.30	15.40	.80	1.70			
<i>Crotalaria intermedia</i> silage	4	27.13	3.30	12.52	8.34	.77	2.20			
Soybean silage, Florida analyses	23	24.64	2.24	10.03	9.08	.74	2.55			
Alfalfa hay ¹	250	91.40	14.90	28.30	37.30	2.30	8.60			
Natal grass hay	1	92.24	3.36	39.49	43.12	1.44	4.83			
Timothy hay, all analyses ¹	221	88.40	6.20	29.80	45.00	2.50	4.90			
Grapefruit refuse, dried	1	91.77	4.94	11.94	69.60	1.06	4.23			
Dried orange peel	1	86.04	5.84	10.64	64.74	.69	4.13			
Beet pulp, dried ¹	48	91.80	8.90	18.90	59.60	.90	3.50			

COEFFICIENTS OF DIGESTIBILITY

Napier grass silage	3	29.07	50.14	40.19	64.70				
Corn silage, well matured ¹	27	51.00	65.00	71.00	82.00				
<i>Crotalaria intermedia</i> silage	4	62.87	32.71	40.57	66.92				
Soybean silage ²	4	55.30	42.90	61.20	48.90				
Alfalfa hay ¹	109	71.00	43.00	72.00	38.00				
Natal grass hay	4	8.16	59.34	51.85	68.64				
Timothy hay	58	48.00	50.00	62.00	50.00				
Grapefruit refuse, dried	4	24.83	71.52	92.43	79.37				
Dried orange peel	4	36.57	93.91	88.51	66.59				
Beet pulp, dried ¹	3	52.00	83.00	83.00					

DIGESTIBLE NUTRIENTS

Napier grass silage		0.34	7.25	5.80	0.44	14.38	1.05	44.16
Corn silage, well matured		1.07	4.10	10.93	.66	17.59	4.07	66.88
<i>Crotalaria intermedia</i> silage		2.08	4.10	3.38	.52	10.72	7.66	39.48
Soybean silage		1.24	4.30	5.57	.36	11.92	5.01	48.20
Alfalfa hay		9.09	12.17	26.86	.87	50.08	9.95	54.79
Natal grass hay		29	23.43	22.96	.99	48.31	.31	52.22
Timothy hay		2.98	14.90	27.00	1.25	48.59	3.37	54.97
Grapefruit refuse, dried		1.23	8.54	64.33	.84	75.99	1.34	82.80
Dried orange peel		2.14	9.99	57.30	.05	69.55	2.49	80.82
Beet pulp, dried		4.63	15.69	49.47		69.79	5.04	76.02

¹ HENRY, W. A., and MORRISON, F. B. See footnote 5.

² HOPKINS, C. G. COMPOSITION AND DIGESTIBILITY OF CORN ENSILAGE, COW PEA ENSILAGE, SOJA BEAN ENSILAGE AND CORN-FODDER. Ill. Agr. Expt. Sta. Bull. 43, pp. 181-208. 1896.

SUMMARY AND CONCLUSIONS

The composition, coefficients of digestibility, and digestible nutrients of Napier grass and *Crotalaria intermedia* silages, Natal grass hay, and dried refuse from grapefruit canneries are presented for the first time. Additional data on dried refuse from orange canneries are given.

Napier grass silage is low in digestible protein and contains approximately two-thirds as much total digestible nutrients as does corn silage.

Crotalaria intermedia silage provides less total digestible nutrients than does corn silage, but it is a better source of protein. Cut in an earlier stage of maturity, this silage probably would have been more desirable.

Natal grass hay is comparable with timothy hay in feeding value.

The dried refuses from grapefruit and orange canneries are lower in digestible crude protein, but slightly higher in total digestible nutrients, than is dried beet pulp. These feeds are comparable.

BACTERIAL LEAF SPOT OF ALFALFA¹

By A. J. RIKER, *professor of plant pathology, Wisconsin Agricultural Experiment Station*; F. R. JONES, *senior pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*; and MARGUERITE C. DAVIS, *research assistant in plant pathology, University of Wisconsin*.

INTRODUCTION

The bacterial leaf spot described here was first recognized on Turkestan and Grimm alfalfa (*Medicago sativa* L.) in two locations at Madison, Wis., in August 1930. The alfalfa in both instances was in cultivated experimental rows and had been repeatedly splashed by watering with a garden hose. In October 1931 the disease recurred under similar circumstances at one of these locations, but with the abandonment of alfalfa growing at these places the disease has not been seen during the three following summers. At no time has it been found in alfalfa fields. These observations, together with the results of inoculations, suggest that while the disease appears potentially destructive, it does not often find favorable conditions for development in this locality. Inasmuch as it may develop elsewhere and may easily be mistaken for other well-known diseases, its description seems warranted.

DESCRIPTION OF THE LEAF SPOT

This leaf spot, like most bacterial leaf spots, is at first very small and water-soaked in appearance. As the spots increase in size, they may coalesce, especially along the midrib and at the ends of the leaflets, forming areas of dead tissue which soon dries. The dry center of spots attaining a diameter of 2 to 3 mm is often yellow with a dark-brown border surrounded by a straw-colored halo. Smaller lesions may appear merely as dark-brown spots. Characteristic leaf injury from this disease is shown in figure 1. No stem lesions have been observed except following inoculations.

Thus, only in the early stages of its development does the disease suggest its bacterial origin. At that time it is easily distinguished from leaf infections of the bacterial stem blight caused by *Phytophthora medicaginis* (Sack.) Bergey et al. by the absence of the stem lesions characteristic of that disease and also by the small size of the spots, which have not been observed to extend to form the large yellow areas described for that disease. After the early water-soaked condition has passed, this leaf spot resembles certain fungous leaf spots. The smaller dark-brown spots are not easily distinguished from partly developed spots caused by *Pseudopeziza medicaginis* (Lib.) Sacc. The larger bordered spots may closely resemble those caused by

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Pseudoplea briosiana (Poll.) Hoehn. Because of the strong resemblance of this bacterial spot to lesions caused by these two fungi, it may have occurred unrecognized many times in the past.

ISOLATIONS AND INOCULATIONS

Six separate isolations of the organism were made in 1930 with the usual poured-plate technic. The pathogenicity of the cultures obtained was proven on plants in the greenhouse, the bacteria were reisolated, and the pathogenicity of the cultures obtained again demonstrated by inoculation.

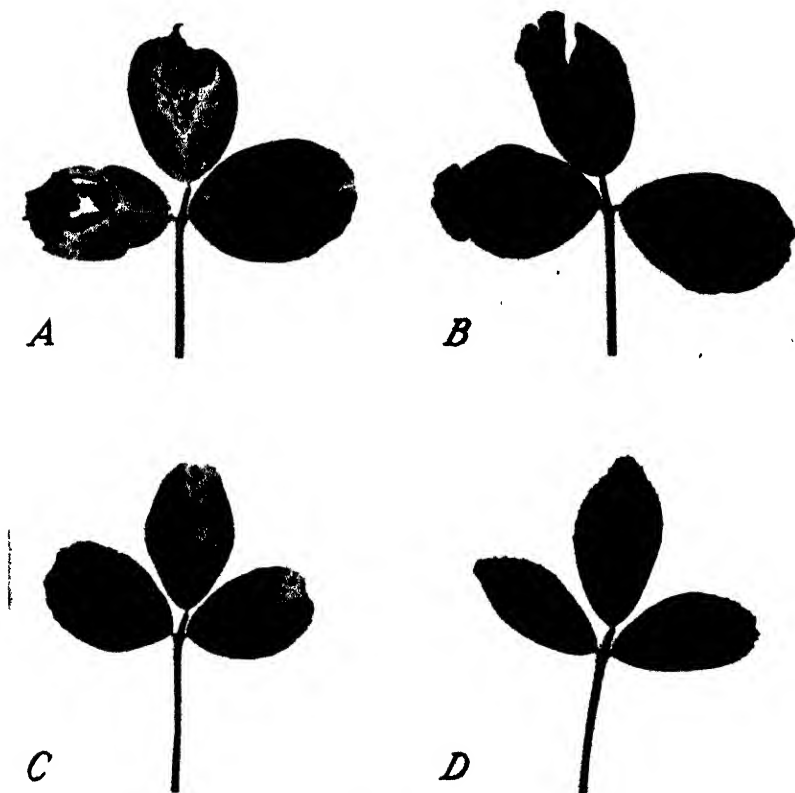


FIGURE 1.—Bacterial leaf spot of alfalfa on diseased leaves from plants growing in a cultivated plot as compared with a healthy leaf: A and B, Leaves showing extensive injury of leaflets chiefly at the ends and along the midribs; C, leaf showing isolated blackened and halo-surrounded spots; D, healthy leaf. (About natural size.)

The plants inoculated were kept in a closed glass chamber for half a day before the leaves were sprayed with the bacterial suspension in distilled water, and were left in the chamber for a half day after this inoculation. In some cases a trace of castile soap was added to the bacterial suspension in order to facilitate wetting the leaves. Under these conditions infection was often far more destructive to the plant than natural infection observed in the field. Puncture inoculations

were also made, which, though successful, were not commonly as satisfactory as those made by spraying the leaves.

The cultures were carried in stock until 1933, when their pathogenicity was again demonstrated by greenhouse inoculations. Following this, single-cell isolations were made from the six different cultures.² The pathogenicity of these single-cell isolations was demonstrated soon after they were made and again after they had been carried in stock for several months.

BACTERIOLOGICAL STUDIES

The bacteriological characters of these six single-cell cultures were determined according to the common procedures. Unless otherwise noted, the methods employed were those given in current (1933) revisions of publications by the Committee of Bacteriological Technic of the Society of American Bacteriologists.³ Each of these tests was made in triplicate with suitable controls for each of the six cultures. Unless otherwise noted, each test was performed at least a second and usually a third time.

MORPHOLOGY OF THE ORGANISM

The organisms were all small rods. They were measured⁴ from smears of 1-day-old cultures grown on yeast-infusion glucose agar at 22° C. The slides were stained with the negative nigrosine preparation. One hundred organisms were measured from each strain. The average of all was 2.14 μ by 0.45 μ . They ranged in length from 0.93 μ to 4.56 μ , and in width from 0.28 μ to 0.77 μ . The organisms were Gram-negative and not acid-fast. No capsules were found after growth for a week on ascitic agar.

Flagella stains with the Caesares-Gil method made on 3 of the 6 strains revealed a single polar flagellum. The other cultures were all observed to be motile in hanging-drop preparations. Some difficulty was experienced in securing motility in these cultures that had been carried in artificial media for a long time.

GROWTH CHARACTERS ON VARIOUS MEDIA

Colony characters determined on nutrient agar after 5 days at room temperature were as follows: Growth, rapid; form, circular; surface, smooth; elevation, convex; edge, entire; internal structure, finely granular; chromogenesis, white to pale yellow; deep colonies, lense-shaped; well-isolated colonies, about 10 to 12 per plate, 7 mm in diameter.

Colony characters determined on plain gelatin after 5 days at room temperature showed the following characters: Growth, rapid; form, circular; edge, erose; liquefaction, cup; internal structure, finely granular. Liquefaction occurred on the fourth day.

Agar strokes on nutrient agar incubated 2 days at room temperature had the following characters: Growth, abundant; form of growth, filiform; elevation, convex; luster, glistening; surface, smooth; odor, absent; chromogenesis, white to pale yellow; consistency, butyrous;

² These isolations were made by Dr. J. A. Pinckard.

³ SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE OF BACTERIOLOGICAL TECHNIC. *MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA*. [Looseleaf, See 1933 supplement.] Geneva, N. Y. 1923.

⁴ The measurements were made by Mary Jacobson.

medium, unchanged; optical character, translucent. Studies made on 2-percent glucose nutrient agar, 2-percent starch agar, and yeast-infusion glucose agar showed the same characters.

Potato plugs incubated at room temperature for 24 hours showed bacterial characteristics resembling those on nutrient agar. The potato plugs were gray in 72-hour-old cultures.

Stabs in plain gelatin were incubated at room temperature. Liquefaction occurred on the fourth day. On the sixth day, the type of liquefaction was crateriform; and on the tenth day, stratiform.

Growth of the organisms was made in 24 hours in nutrient broth. The medium was slightly cloudy; a small amount of sediment was formed which was viscid on agitation. There was no odor. The same was true of organisms grown in 2-percent glucose broth.

The diastatic action of the organisms was determined on 0.2 percent starch agar. When abundant growth appeared, the plates were flooded with a saturated iodine solution in 50-percent alcohol. Clear zones appeared measuring about 7 mm, indicating diastatic action. This is particularly interesting in relation to the results from sugar fermentation.

Transfers made to litmus milk and kept at room temperature showed the following:

2 days, no color change; 2-mm serum zone.

4 days, no color change; 6-mm serum zone.

7 days, change in color from lavender to tan (with the exception of two organisms, lavender to brown); 15-mm serum zone.

10 days, same color change as on seventh day; 15-mm serum zone.

14 days, entirely clear except for sediment.

Transfers were spread on milk-agar plates (nutrient agar with 1 cc litmus milk) and incubated at room temperature for 2 days. Giant colonies were formed, surrounded by clear zones. These plates were flooded with 10-percent acetic acid. No precipitation occurred in the clear zones, indicating that digestion had taken place.

Nitrate broth was seeded and kept at room temperature. Readings were made after 1, 2, 4, 7, and 10 days for ammonia, nitrites, and nitrates. Growth was obtained in 48 hours. Tests with Nessler's reagent showed the presence of ammonia on the fourth day. More positive reactions for ammonia were obtained on the seventh and tenth days. Tests with Trommsdorf's reagent were negative for nitrites; and tests made with diphenylamine showed that nitrates were still present. Cultures in nitrate broth were uniformly cloudy; sediment formed was viscid on agitation; odor, absent.

Studies were made of sugar fermentation in a yeast-infusion mineral-salt medium with the following composition: Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.2 g; dipotassium phosphate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$), 0.2 g; sodium chloride (NaCl), 0.2 g; calcium chloride (CaCl_2), 0.1 g; yeast infusion (20 percent), 50 cc; carbon source, 5 g; and distilled water, 950 cc. The reaction was adjusted to pH 6.8. A neutral solution of the carbon source was sterilized separately and added aseptically. The organisms were grown for 7 days at room temperature in this medium, with, respectively, 0.5 percent of pectin, lactose, dextrin, mannitol, sucrose, maltose, glycerin, levulose, salicin, starch, galactose, inulin, and glucose. Determinations of the hydrogen-ion concentration were then made with the common quinhydrone equipment. The results of one representative series of tests appear in

table 1. Some acid was found only with the galactose medium and even this was within the range which carbon dioxide might induce. The reaction commonly became slightly alkaline, doubtless because of utilization by the bacteria of the yeast infusion. The growth developed suggests that the various materials were employed with the formation of relatively neutral products. The action on starch in relation to the action on various sugars is noteworthy. For the purposes of the present study, quantitative measure of sugar utilization and of carbon dioxide production did not appear necessary.

TABLE 1.—*Summary of reactions induced by various single-cell strains of alfalfa leaf spot bacteria incubated 7 days at room temperature in yeast-infusion mineral-salt liquid media containing stated sources of carbon*¹

Substance tested	Hydrogen-ion concentration from strain no —						
	1	2	3	4	5	6	Control
d-fructose.....	7.2	7.0	7.0	7.1	7.1	7.1	6.6
d-galactose.....	6.5	6.4	6.6	6.3	6.6	6.7	6.7
d-glucose.....	6.9	6.8	7.0	6.5	6.8	6.7	6.4
Sucrose.....	7.3	7.4	7.1	7.2	7.2	7.2	6.8
Maltose.....	7.3	7.3	7.2	7.2	7.3	7.2	7.1
Lactose.....	7.2	7.1	7.1	7.3	7.3	7.3	6.5
Starch.....	6.7	6.7	6.7	6.7	6.8	6.7	6.7
Inulin.....	7.4	7.4	7.3	7.5	7.4	7.4	6.6
Dextrin.....	7.5	7.2	7.3	7.3	7.3	7.2	7.1
Pectin.....	7.0	7.0	6.8	7.0	7.1	7.1	6.5
Glycerol.....	7.2	7.4	7.1	7.3	7.4	7.3	6.6
Mannitol.....	7.5	7.5	7.5	7.5	7.5	7.5	7.1
Salicin.....	7.3	7.4	7.3	7.5	7.4	7.4	6.7
None.....	7.3	7.4	7.4	7.4	7.5	7.6	6.6

¹ There was no growth in the unseeded controls and slight growth in media containing no source of carbon except the yeast infusion. In the starch medium, turbidity appeared only in the upper 10 to 12 mm of the medium. In all other cases relatively dense turbidity and compact sediment, viscid on agitation, indicated good growth. Two other trials gave comparable results.

The suitable temperature for growth was determined with nutrient-agar stroke cultures incubated at the following temperatures: 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C. These cultures were incubated for 1 week, with the following results: At 4° and 36°, scanty growth; at all other temperatures, abundant growth. In a repetition of the test after incubation for 2 days the results were: No growth at 4°, 8°, and 36°; scant growth at 12° and 16°; and moderate growth at 20°; abundant growth at 24°, 28°, and 32°. In 7 days the results were: No growth at 36°; scant growth at 4° and 8°; abundant growth at 12°, 16°, 20°, 24°, 28°, and 32°. These studies indicate that the most rapid growth of these bacteria on nutrient agar is made between 24° and 32°.

Nutrient-agar shake cultures were used to study the oxygen requirements of the organisms. A suspension of the organism was prepared and three successive loop dilutions of the suspension were made in successive tubes. A small amount of the agar shake cultures from tubes 1, 2, and 3, respectively, was drawn up into different sterile capillary tubes, leaving a clear space at each end which was sealed. Three capillary-tube cultures and three test-tube agar shake cultures with suitable controls were made for each organism and incubated at room temperature. After 10 days colonies showed only in the upper layer of medium in the test tubes; and only at the ends in the capillary tubes, indicating that the organisms are aerobes.

The characters of this alfalfa leaf spot organism do not appear similar to those given in any description found for a bacterial plant pathogen. When compared with *Phytomonas medicaginis*, in addition to the difference in symptoms induced, there appear important bacteriological differences. For example, in contrast with the characters given in this paper, Sackett⁵ reported that *P. medicaginis* was doubtful in relation to the Gram stain, made grayish-white growth on agar, did not liquefy gelatin, did not digest starch, did not form ammonia from nitrate, and produced no change in litmus milk except to make it blue.

TECHNICAL DESCRIPTION

This organism is named and briefly characterized as follows:

***Phytomonas alfalfae*, n. sp.⁶**

Organism a rod, average $2.14\ \mu$ by $0.45\ \mu$, motile by one flagellum, Gram-negative, not acid-fast, apparently without spores or capsule. On nutrient agar after 5 days at room temperature colony characters were: Growth, rapid; form, circular; surface, smooth; elevation, convex; edge, entire; internal structure, finely granular; chromogenesis, white to pale yellow; well-isolated colonies, 7 mm in diameter; deep colonies, lense-shaped. Gelatin liquefied. Trace of acid formed in yeast-infusion mineral-salts medium with galactose. No change or an alkaline reaction with 12 other sources of carbon. Starch hydrolyzed. Ammonia formed slowly in a nitrate medium. Litmus milk cleared in 2 weeks with digestion of the casein. No growth where atmospheric oxygen was excluded. Growth at temperatures between 4° and 32° C.; most rapid growth between 24° and 32° . Organism causes leaf spots on alfalfa.

SUMMARY

A bacterial leaf spot of alfalfa, apparently new to science, has been observed at Madison, Wis. The symptoms and isolation and inoculation studies are described. On the basis of the morphological and physiological characters of single-cell isolations from six cultures of the organism, it is described as a new species and the name *Phytomonas alfalfae* is suggested.

⁵ SACKETT, W. G. A BACTERIAL DISEASE OF ALFALFA. Colo. Agr. Expt. Sta. Bull. 158, 32 pp., illus. 1910.

⁶ Synonyms according to other systems of classification in use among plant pathologists are *Pseudomonas alfalfae* and *Bacterium alfalfae*.

CALCIUM CYANAMIDE IN RELATION TO CONTROL OF CLUBROOT OF CABBAGE¹

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INTRODUCTION

In a previous publication³ the writers reported comparative studies of the effect of calcium hydrate ($\text{Ca}(\text{OH})_2$), calcium carbonate (CaCO_3), and certain other compounds, when applied to infested soil, upon infection of cabbage by the clubroot organism (*Plasmodiophora brassicae* Wor.). It was shown that, in well-watered soil in the greenhouse, infection was reduced perceptibly when the reaction of an acid soil was changed to about pH 7.0, and it was usually inhibited completely at pH 7.2 and above. In the field even heavier applications of $\text{Ca}(\text{OH})_2$ and CaCO_3 often failed to control the disease although the reaction of the soil was maintained at pH 7.0 or above. When soil was removed from such field plots, however, and tested by growing cabbage plants upon it in the greenhouse, inhibition of the disease was usually complete. The difference between field and greenhouse environments, therefore, appeared to influence the effectiveness of these materials upon clubroot development.

The present paper is a report of a comparative study of $\text{Ca}(\text{OH})_2$ and calcium cyanamide (CaCN_2) upon clubroot infection. When applied to moist soils CaCN_2 undergoes hydrolysis as a result of which $\text{Ca}(\text{OH})_2$ and urea ($\text{CO}(\text{NH}_2)_2$) are formed. Before hydrolysis is complete, the cyanamide anion (CN_2) may be toxic to higher plants. Cyanamide disappears from the soil solution more slowly in some soils than in others, and the factors which influence this change have been studied recently by Fink⁴. In normal soils urea is ammonified rapidly and at this stage a marked increase in alkalinity or decrease in acidity results because two basic substances, $\text{Ca}(\text{OH})_2$ and $(\text{NH}_4)_2\text{CO}_3$, have been produced. When nitrification of the latter takes place, there should be no change in reaction because there is sufficient Ca in the CaCN_2 to combine with the nitric acid formed. The extent of the influence of the addition of CaCN_2 to a soil on the reaction of that soil will thus depend on soil conditions and the time that has elapsed since the addition. In the case of very acid soils, a marked decrease in acidity should result for some time, because the soil acids will combine with the free lime and ammonia as soon as they are

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² The authors are indebted to Prof. Emil Truog, Department of Soils, University of Wisconsin, for helpful suggestions.

³ LARSON, R. H., and WALKER, J. C. SOIL TREATMENT IN RELATION TO CLUBROOT OF CABBAGE. Jour. Agr. Research 48:749-759. 1934.

⁴ FINK, D. S. SOIL FACTORS WHICH PREVENT TOXICITY OF CALCIUM CYANAMIDE. Jour. Amer. Soc. Agron. 26: 929-939, illus. 1934.

formed and hold on to the ammonia with considerable tenacity, thus slowing up its further change to nitric acid.

The fact that CaCN_2 when applied as a nitrogen fertilizer reduces acidity or increases alkalinity of the soil has led to an interest in its value as a clubroot inhibitor. Both greenhouse and field experiments were carried out to determine its effectiveness on southern Wisconsin soils.

GREENHOUSE EXPERIMENTS

Two series of greenhouse pot tests were conducted. Naturally infested Carrington silty clay loam soil from the Miller farm, Kenosha County, Wis., was used in 2-gallon earthenware crocks. Six kilograms of soil were weighed out for each crock and the desired amount of chemical was thoroughly mixed with the soil. The rates of application are given on the basis of pounds per surface acre; 0.385 g per crock was approximately equal to 100 pounds per acre. Since the cyanamide anion (CN_2) is toxic to plants, it is necessary to delay planting for a sufficient period after the application of CaCN_2 to allow complete hydrolysis and the removal of CN_2 from the soil solution. Cabbage (*Brassica oleracea capitata*) seedlings were transplanted to the crocks 2 weeks after treatment. The soil moisture was kept at about 75 percent of the water-holding capacity for 5 weeks, after which period the plants were removed and examined.

In the greenhouse experiments $\text{Ca}(\text{OH})_2$ and $\text{CO}(\text{NH}_2)_2$ were included. The amounts applied were so arranged as to compare a given amount per acre of CaCN_2 with (1) the approximate amount of $\text{Ca}(\text{OH})_2$ it would yield upon hydrolysis in the soil; (2) the approximate amount of $\text{CO}(\text{NH}_2)_2$ it would yield; and (3) those amounts of $\text{Ca}(\text{OH})_2$ and $\text{CO}(\text{NH}_2)_2$ applied together. Thus, for example, 125 pounds of CaCN_2 yields, when completely hydrolyzed, 87.5 pounds of $\text{Ca}(\text{OH})_2$ and 62.5 pounds of $\text{CO}(\text{NH}_2)_2$.

The results of the first series are given in table 1. The plants in the untreated soil were all infected. As increasing amounts of $\text{Ca}(\text{OH})_2$ were added, infection was gradually reduced until none occurred at 525 pounds per acre or more. When $\text{CO}(\text{NH}_2)_2$ was added at the rate of 125 pounds per acre together with $\text{Ca}(\text{OH})_2$ at the rate of 175 pounds, no infection occurred; while in the pots containing $\text{Ca}(\text{OH})_2$ at the rate of 175 pounds without urea, 66.7 percent infection occurred. It was evident, therefore, that urea, probably through its rapid conversion to $(\text{NH}_4)_2\text{CO}_3$, had a decidedly toxic effect upon *Plasmodiophora brassicae*. Urea was not used alone in this series. CaCN_2 was completely inhibitive at 250 pounds and above.

In the second series (table 1), smaller applications were included and various amounts of urea alone were used. Again complete inhibition with $\text{Ca}(\text{OH})_2$ was not attained below 525 pounds per acre. No infection resulted when 250 pounds per acre or more of $\text{CO}(\text{NH}_2)_2$ was applied. The pronounced effect of urea is shown when $\text{Ca}(\text{OH})_2$ alone and $\text{Ca}(\text{OH})_2$ plus urea are compared. $\text{Ca}(\text{OH})_2$ at 175 pounds permitted 66.7 percent infected plants; $\text{CO}(\text{NH}_2)_2$ at 125 pounds permitted 29.2 percent; the two combined in the same respective amounts permitted 20.8 percent i. e., approximately the same amount as urea alone. $\text{Ca}(\text{OH})_2$ at 350 pounds allowed 33.3 percent infected plants; $\text{CO}(\text{NH}_2)_2$ at 250 pounds, no infection; the two applied together resulted in no infection.

TABLE 1.—*The relative effect of calcium hydrate, urea, and calcium cyanamide upon infection of cabbage by Plasmodiophora brassicae when applied to the soil under greenhouse conditions; series 1 and 2*

SERIES 1

Chemical applied	Quantity	Plants tested	Plants diseased	Chemical applied	Quantity	Plants tested	Plants diseased
	Pounds per acre	Number	Percent		Pounds per acre	Number	Percent
None.....	175	60	100	CO(NH ₂) ₂	375	30	0
	350	30	66.7	Ca(OH) ₂	700	30	0
Ca(OH) ₂	525	30	23.3	CO(NH ₂) ₂	500	30	0
	700	30	0	Ca(OH) ₂	1,050	30	0
	1,050	30	0	CO(NH ₂) ₂	750	30	0
Ca(OH) ₂	175	30	0		250	30	0
CO(NH ₂) ₂	125	30	0	CaCN ₂	500	30	0
Ca(OH) ₂	350	30	0		750	30	0
CO(NH ₂) ₂	250	30	0		1,000	30	0
Ca(OH) ₂	525	30	0		1,500	30	0

SERIES 2

Chemical applied	Quantity	Soil reaction	Plants tested	Plants diseased	Chemical applied	Quantity	Soil reaction	Plants tested	Plants diseased
	Pounds per acre	pH	Number	Percent		Pounds per acre	pH	Number	Percent
None.....	87.5	6.4	72	100.0	Ca(OH) ₂	175.0	6.6	24	20.8
	175.0	6.4	24	83.3	CO(NH ₂) ₂	125.0	6.6	24	0
Ca(OH) ₂	350.0	6.6	24	66.7	Ca(OH) ₂	350.0	6.8	24	0
	525.0	6.8	24	33.3	CO(NH ₂) ₂	250.0	6.8	24	0
	62.5	7.0	24	0	Ca(OH) ₂	525.0	6.8	24	0
	125.0	6.4	24	58.3	CO(NH ₂) ₂	375.0	6.8	24	0
CO(NH ₂) ₂	250.0	6.6	24	29.2		1,050.0	7.2	24	0
	375.0	6.8	24	0	Ca(OH) ₂	750.0	6.7	24	12.5
	1,000.0	6.8	24	0	CO(NH ₂) ₂	500.0	6.8	24	0
Ca(OH) ₂	87.5	7.2	24	0		750.0	7.1	24	0
CO(NH ₂) ₂	62.5	6.6	24	62.5	CaCN ₂	500.0	7.3	24	0

¹ At the close of the experiment.

The next point of interest is the comparison of CaCN₂ with the combined effect of its products of hydrolysis, Ca(OH)₂ and CO(NH₂)₂. It has been pointed out that before hydrolysis is complete the CN₂ anion is toxic to higher plants. Nothing is known of its toxicity to the clubroot organism, but it may be expected that some reduction in the infestation might occur because of the presence of CN₂ before complete hydrolysis. That such is the case is indicated in the results. CaCN₂ at 125 pounds reduced infection to 12.5 percent, while the proportionate amounts of Ca(OH)₂ (87.5 pounds) and CO(NH₂)₂ (62.5 pounds) applied together reduced it to only 62.5 percent. CaCN₂ at 250 pounds reduced infection to nil, while the proportionate amounts of Ca(OH)₂ (175 pounds) and CO(NH₂)₂ (125 pounds) applied together, permitted 20.8 percent infection.

It is indicated in these experiments that CaCN₂ is approximately equal to urea and about twice as effective as Ca(OH)₂ in controlling clubroot. Specifically, 250 pounds of CaCN₂, 250 pounds of CO(NH₂)₂, and 525 pounds of Ca(OH)₂ each completely inhibited infection.

FIELD EXPERIMENTS

Field trials were conducted in 1933 and 1934 at Franksville, Wis., and on the Miller farm near Somers. The soil at both of the locations has been previously described.⁵

The first series at the Franksville field (referred to as series 1) was on part of the plot used in former experiments. A portion of this area had received 2½ tons of Ca(OH)₂ in the autumn of 1930, but little or no control of clubroot was secured in the following year. The entire area received an application of 220 pounds of CaCN₂ per acre in November 1932. It was divided into nine parts, each 9 rods by 1 rod in dimension. To certain of these nine plots CaCN₂ was applied on May 24, 1933. To other plots Ca(OH)₂ was applied on June 26, 1933. In this and subsequent field experiments the materials were incorporated into the soil by very thorough disking immediately following application. The soil was disked and dragged again just before transplanting. The entire area was planted on July 11. No fertilizer other than CaCN₂ was applied. On September 28, plants were pulled and the amount of clubroot recorded. The same area was planted in 1934 without any further addition of lime or cyanamide. The results are given in table 2.

TABLE 2.—*The effect of various amounts of calcium hydrate and calcium cyanamide on clubroot infection in the field; Franksville series 1*

Chemical applied	Quantity	1933 results			1934 results		
		Soil reaction ¹	Plants examined	Plants diseased	Soil reaction ¹	Plants examined	Plants diseased
	<i>Pounds per acre</i>	<i>pH</i>	<i>Number</i>	<i>Percent</i>	<i>pH</i>	<i>Number</i>	<i>Percent</i>
None.....		6.2	170	79.4	6.8	100	65
	220	6.2	167	64.1	6.5	100	94
	440	6.2	183	63.9	6.5	100	88
CaCN ₂	1 440	6.6	151	21.2	7.2	100	34
	880	6.3	174	86.2	6.6	100	56
	1 880	6.2	160	8.0	7.2	111	27
	2 000	6.4	158	22.2	7.0	100	28
Ca(OH) ₂	3 000	6.8	171	24.6	7.0	100	51
	4 000	7.0	185	16.8	8.0	100	18

¹ At the end of the growing season.

² These plots had received an application of 2½ tons of Ca(OH)₂ in the fall of 1930.

As pointed out earlier⁵ with Ca(OH)₂, the behavior of neutralizing substances as clubroot inhibitors in the greenhouse is no indication of their success in the field. The untreated soil in this series was essentially the same in reaction as that used in the greenhouse experiments. Five hundred and twenty-five pounds of Ca(OH)₂ per acre was sufficient to completely check clubroot in the greenhouse, but 4,000 pounds in the field still permitted some infection. Two hundred and fifty pounds per acre of CaCN₂ in the greenhouse prevented infection, but 880 pounds in the field did not inhibit the parasite completely.

Ca(OH)₂ at the rate of 4,000 pounds per acre gave good control in 1933 and in 1934. Three thousand pounds reduced infection fairly well in 1933, but this plot showed a decided increase in infection in 1934. Control on the 2,000-pound plot was nearly as good as on the

⁵ LARSON, R. H., and WALKER, J. C. See footnote 2.

4,000-pound plot, but the former had received an application of $\text{Ca}(\text{OH})_2$ in 1930.

CaCN_2 was commercially effective only in the 440- and 880-pound applications on plots where $\text{Ca}(\text{OH})_2$ had been applied in 1930. Where lime had not been applied previously, the CaCN_2 plots showed high percentages of clubroot infection in both 1933 and 1934.

In 1934, 22 plots, 2 by 3 rods in dimension, were laid out in the Franksville field (series 2). They are listed in table 3 in their order of position, and the type of treatment in each is indicated. The soil reaction in each plot was determined before any treatment was made and again after the crop was harvested. CaCN_2 and $\text{Ca}(\text{OH})_2$ were applied on May 4. At the time of planting, on June 21, 0-9-27 fertilizer was applied in the row at the rate of 300 pounds per acre. Favorable weather prevailed immediately after transplanting, but the following 2 months were decidedly deficient in rainfall. Growth was therefore very slow and the midseason variety used, Marion Market, produced small heads. After harvest (Sept. 11) 100 plants in each plot were pulled and examined for the occurrence of the disease on the roots.

TABLE 3.—*The effect of various amounts of calcium hydrate and calcium cyanamide on clubroot infection in the field; Franksville series 2, 1934*

INDIVIDUAL PLOTS						
Plot no.	Chemical applied	Quantity	Soil reaction		Clubroot-infected plants	Yield per acre
			Before application	After harvest		
		Pounds per acre	pH	pH	Percent	Tons
1.....	None.....	-----	6.2	6.2	69	4.0
2.....	$\text{Ca}(\text{OH})_2$	1,500	6.2	7.4	36	4.0
3.....	CaCN_2	200	6.2	6.5	32	4.1
4.....	None.....	-----	6.1	6.2	50	4.1
5.....	CaCN_2	400	6.2	7.0	66	5.3
6.....	do.....	800	6.8	7.4	58	5.1
7.....	None.....	-----	7.0	6.8	64	5.5
8.....	$\text{Ca}(\text{OH})_2$	1,500	5.9	7.6	59	5.2
9.....	CaCN_2	200	6.2	6.8	55	4.5
10.....	None.....	-----	6.3	6.2	72	5.9
11.....	CaCN_2	400	6.2	6.7	44	6.7
12.....	do.....	800	6.1	7.2	29	5.5
13.....	None.....	-----	6.1	6.2	54	5.1
14.....	$\text{Ca}(\text{OH})_2$	1,500	6.1	7.6	50	5.5
15.....	CaCN_2	200	6.8	6.7	74	7.1
16.....	None.....	-----	6.6	6.6	80	5.7
17.....	CaCN_2	400	6.3	7.0	57	4.8
18.....	do.....	800	6.1	7.2	32	4.4
19.....	$\text{Ca}(\text{OH})_2$	1,500	9.9	7.8	42	5.5
20.....	CaCN_2	200	6.2	6.7	76	4.8
21.....	None.....	-----	6.8	6.5	55	4.0
22.....	CaCN_2	400	6.1	6.8	65	4.0

AVERAGES FOR NUMBER OF PLOTS INDICATED

7.....	None.....	-----	6.4	6.4	63.4	4.90
4.....	$\text{Ca}(\text{OH})_2$	1,500	6.3	7.6	46.8	5.05
4.....	CaCN_2	200	6.4	6.7	59.3	5.13
4.....	do.....	400	6.2	6.9	58.0	5.20
3.....	do.....	800	6.3	7.3	39.7	5.00

Clubroot was by no means held in check completely in any of the plots. There was considerable variation between duplicate plots of the same treatment. The untreated plots varied from 50 to 80

percent in the number of diseased plants; the $\text{Ca}(\text{OH})_2$ treatment varied from 36 to 59 percent; the 200-pound CaCN_2 treatment from 32 to 76 percent; the 400-pound CaCN_2 treatment from 44 to 66 percent; and the 800-pound CaCN_2 treatment from 29 to 58 percent. The variability rather detracts from the value of the differences between the averages for each treatment. Certainly there is no significant difference between the 200- and 400-pound treatments of CaCN_2 and the control. The hydrate treatment and the 800-pound cyanamid treatment changed the soil reaction to alkaline and seem to have reduced infection distinctly although not by any means completely.

The yields from the various plots show no consistently significant differences between treatments. This may be due in part to the poor conditions for growth.

The third trial in 1934 was on the Miller farm. Plots were laid out in a portion of the field which was known to have shown clubroot when it last grew cabbage several years before (about 1930). At that time it had received an application of 1,000 pounds of $\text{Ca}(\text{OH})_2$ per acre. In the fall of 1933, 20 loads of manure per acre were applied. CaCN_2 was applied on May 12 and disked in thoroughly. At the time of planting, on July 10, 125 pounds of 3-12-12 fertilizer per acre were applied in the row. Wisconsin All Seasons, a late yellows-resistant sauerkraut variety, was used.

As a result of the low rainfall in July and August, growth was very slow. During September and October precipitation was fairly abundant. The remainder of the growing season, however, was not long enough and at harvest on October 31 many heads were still soft and immature. On this date a 6-rod strip in the central three rows of each plot was harvested and weighed. Fifty plants in each were pulled and examined for disease. In table 4 the order of the plots in the field is maintained. The reaction of the soil originally was about the same as that of the Franksville soil. The 825-pound treatment was the only one which brought the soil to neutrality. There is rather conclusive evidence here that the two heaviest treatments reduced clubroot infection materially. As at Franksville, however, there was no measurable benefit of calcium cyanamide in increasing yield.

TABLE 4.—*The effect of various amounts of calcium cyanamide on clubroot infection in the field; Miller series, 1934*

Plot no.	Rate of CaCN_2 application	Soil reaction		Club-root infected plants	Yield per acre	Plot no.	Rate of CaCN_2 application	Soil reaction		Club-root infected plants	Yield per acre
		Before application	After harvest					Before application	After harvest		
	Pounds per acre	pH	pH	Percent	Tons		Pounds per acre	pH	pH	Percent	Tons
1.....	None	6.1	6.2	62	7.4	5.....	None	6.0	6.2	30	14.2
2.....	200	6.3	6.5	44	11.5	6.....	825	6.1	7.0	6	12.7
3.....	None	6.2	6.3	56	13.7	7.....	None	6.1	6.2	65	8.8
4.....	400	6.0	6.7	14	15.9						

DISCUSSION

The potential value of CaCN_2 in the control of clubroot can be estimated best from the greenhouse pot tests since the conditions

under which they were conducted probably approach the optimum both for the activity of the parasite and for hydrolysis of the chemical. In these tests CaCN_2 at the rate of 125 pounds per acre was somewhat more effective than $\text{Ca}(\text{OH})_2$ at the rate of 350 pounds per acre. CaCN_2 at 250 pounds completely inhibited infection, while this degree of control by $\text{Ca}(\text{OH})_2$ was not reached until 525 pounds per acre was applied.

A given amount of CaCN_2 was decidedly more effective than the corresponding amounts of $\text{Ca}(\text{OH})_2$ and $\text{CO}(\text{NH}_2)_2$ derived from its hydrolysis. This fact suggests that CaCN_2 affects the clubroot organism not only through the basic substances formed from it but also through the toxic effect of the CN_2 anion in the soil solution before hydrolysis is complete.

Under field conditions in southern Wisconsin in 1933 and 1934 much heavier applications of CaCN_2 and of $\text{Ca}(\text{OH})_2$ did not completely control clubroot even though the materials were worked into the soil quite thoroughly. As earlier pointed out in comparative greenhouse and field experiments with $\text{Ca}(\text{OH})_2$, field environment appears to influence greatly the effectiveness of CaCN_2 as a clubroot inhibitor. Where $\text{Ca}(\text{OH})_2$ had been applied to the soil 3 or 4 years previously, 400 to 800 pounds of CaCN_2 per acre was fairly effective (Franksville series 1 and Miller series), although complete control was not attained. The results show, however, that CaCN_2 has some value and from the limited results obtained in the field and greenhouse it appears to be about twice as effective pound for pound as $\text{Ca}(\text{OH})_2$. Where a nitrogen fertilizer is needed, it can be used together with lime as a corrective for soil acidity with the expectation that conditions for clubroot infection will be made less favorable, and the completeness of control will be influenced by the soil environment.

SUMMARY

A study was made of the influence upon cabbage-root infection of CaCN_2 when applied to clubroot-infested soil.

In greenhouse tests with soil having a reaction of pH 6.4, CaCN_2 at the rate of 250 pounds per acre prevented infection while 525 pounds of $\text{Ca}(\text{OH})_2$ was required to accomplish the same effect.

When CaCN_2 was compared with corresponding amounts of $\text{Ca}(\text{OH})_2$ and $\text{CO}(\text{NH}_2)_2$ resulting from its hydrolysis, the fungicidal value of CaCN_2 was greater, indicating that its toxicity is due not only to the basic substances formed from it, but also to the CN_2 anions in the soil solution before hydrolysis is complete.

Much higher quantities of CaCN_2 were required to reduce infection in the field than in the greenhouse, which is similar to results secured with $\text{Ca}(\text{OH})_2$ earlier and in this series of experiments. This difference is interpreted as due to the influence of soil environment upon the effectiveness of both of these chemicals.

The results in the greenhouse and in the field indicate that CaCN_2 is roughly about twice as effective, pound for pound, as $\text{Ca}(\text{OH})_2$ in reducing the amount of clubroot infection.

In cases where soil acidity needs to be corrected in order to reduce clubroot infection, CaCN_2 can be used up to the point where the need for available nitrogen is satisfied. If a still larger amount of a neutralizing element is required, $\text{Ca}(\text{OH})_2$ should be used to supplement the cyanamide.

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A PHYSIOLOGICAL STUDY OF CRACKING IN STAYMAN WINESAP APPLES¹

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INTRODUCTION

Cracking of fleshy fruits while still attached to the parent plant occurs in many cultivated species. In sweet cherry, apple, plum, peach, grape, strawberry, and some citrus fruits this kind of injury is sometimes so extensive as to result in great economic loss to growers. Rapid deterioration usually follows the exposure of the ruptured tissues to the air, and the injured fruit becomes worthless or of inferior grade. Losses of this nature amounting to as much as 75 percent have been observed by the writer in commercial orchards of sweet cherries, apples, and plums. Many fruits, on the other hand—such as sour cherry and some varieties of apple—seldom exhibit this phenomenon. In many fruits there are wide varietal differences in the extent to which cracking occurs under apparently similar environmental conditions.

The present paper deals primarily with cracking of the skin and underlying tissue in the fruit of the Stayman Winesap apple. Most of the observations and experiments were made in 1932-33 at the West Virginia University Experiment Farm at Kearneysville, and in 1933-34 at the Laboratory of Plant Physiology of the Johns Hopkins University.

In this study cracking has been observed extensively in only two varieties of apples, the Stayman Winesap and the York Imperial. In the Stayman Winesap cracking occurs largely on the cheeks of the fruit in the form of irregular breaks in the skin and underlying flesh (fig. 1). Individual cracks vary from almost invisible short slits to cracks several millimeters deep that extend in an approximately horizontal plane entirely around the fruit. There is no marked predominance of cracks in any particular plane in relation to the long axis of the fruit. Late in the growing season cracks originating near the fruit stem and extending outward in nearly straight meridional lines towards the cheeks are commonly observed. Cracks around the calyx basin are rare. In the York Imperial, cracks originating

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² For many helpful suggestions relating to these experiments, especially with reference to evaporativity as a climatic feature and to water relations in general, and for much help in interpreting the results and presenting them in this form, the writer wishes to express his appreciation to Prof. Burton E. Livingston, director of the Laboratory of Plant Physiology of the Johns Hopkins University. Helpful suggestions were received also from Dr. H. E. Knowlton, head of the Horticulture Department of West Virginia University; and acknowledgment is due Dr. A. B. Groves, of the Winchester Research Laboratory, Winchester, Va., for taking the photographs used in figure 1.

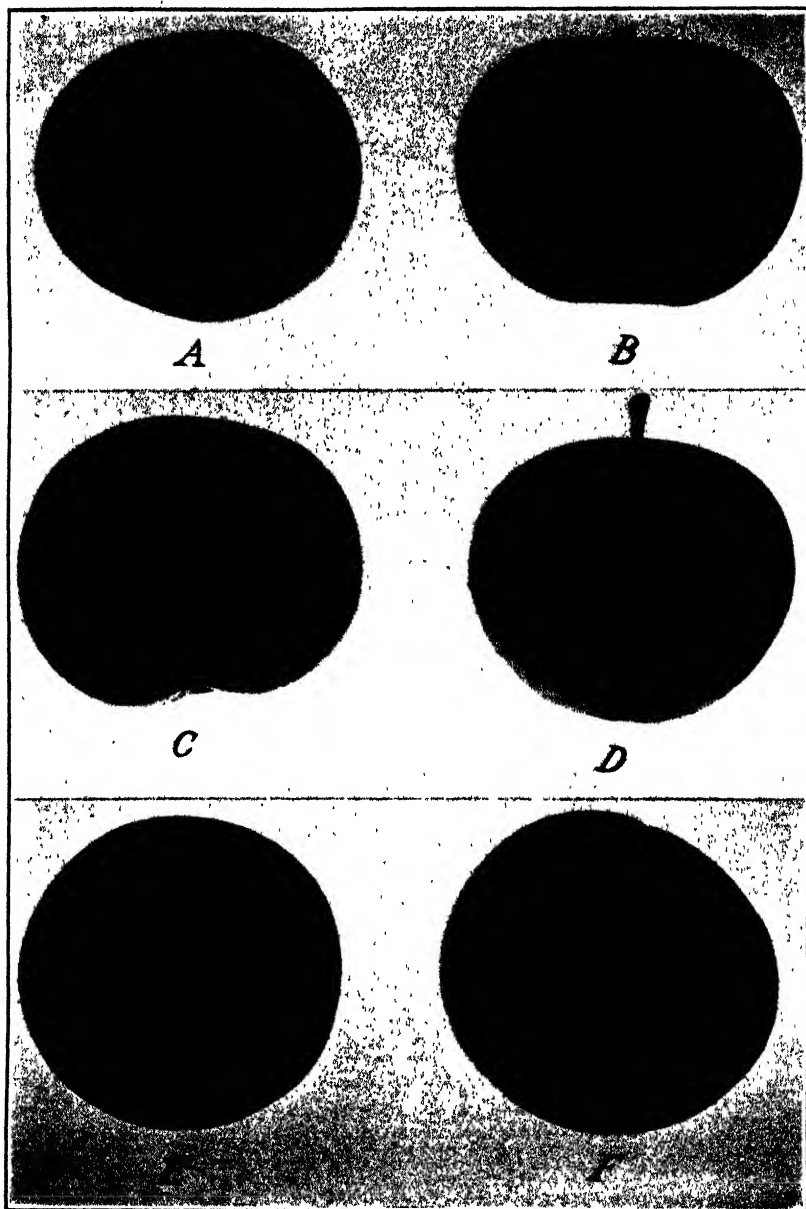


FIGURE 1.—Characteristic cracks in Stayman Winesap apples: *A*, Healed crack; *B*, *D*, and *E*, cracks originating in regions of russet; *C*, cracks originating in regions deformed by apple scab lesions; *F*, cracks originating in or near the stem depression.

at the calyx and extending like meridians toward the cheeks have been predominant in several hundred cracked specimens examined. Cracks in the stem cavity are also common in this variety, but on the cheeks of the fruit they are of much less frequent occurrence in the York Imperial than in the Stayman Winesap. In many specimens of Stayman Winesap, and in a few specimens of York Imperial, cracks that originated early in the season, when the apple was not nearly full grown, usually healed by suberization.

REVIEW OF LITERATURE

It has commonly been stated or implied that cracking of fruits is caused by a great and sudden increase in the water content of the soil, and that maintenance of a nearly constant soil-moisture content about the tree roots throughout the season of fruit growth should prevent cracking or greatly limit its severity. Discussing water relations of deciduous fruits in general, Chandler (8, p. 165)³ states that "Certain injuries, such as cracking of the fruit, may result from a heavy irrigation late in its development, if growth has been checked by lack of water earlier."

Gardner, Bradford, and Hooker (12, p. 83) say that splitting is "most likely to occur shortly before maturity when rains follow a period of drought during which the fruit has been checked in its growth * * *. Heavy, late irrigation following a long dry season has the same effect." These writers suggest that a previous retardation of growth may render the fruit skin less able to expand rapidly in response to increased pressure within, thus predisposing the fruit to cracking when the growth rate is suddenly accelerated.

According to Heald (17, pp. 101-101):

The rupturing of nearly mature, soft-skinned fruits, such as cherries, plums or tomatoes, when a rain follows a rather prolonged dry period is a fairly common phenomenon. These troubles have been proved to result from high sap pressure due to excessive water supply.

It is not clear whether the term "sap pressure" as used here refers to the vacuolar contents of the living cells in the region of the crack or to the contents of the tracheae which supply water to these cells; the causes of excessive pressure in the two cases might be very different.

Coit (9) discusses in some detail the development of splits in citrus fruits, with special reference to the navel orange. He says that these ruptures occur most frequently in the navel but are also found on the sides of the fruit, where they are associated with terratological cavities or "seams" in the skin. Regarding the causes of splitting in oranges, Coit states (9, p. 328):

The most common theory in regard to the cause of splits is that an irregular water supply causing wide variations in the moisture content of the soil, produces a greater fluctuation in the growth of the interior than in the skin of the orange. Such a theory is quite reasonable, but such a cause should be regarded as contributory only, inasmuch as only a part of the fruit on any given tree will split.

Fawcett and Lee (11) point out that splits in citrus fruits are commonly associated with diseased tissues, such as lesions due to *Alternaria citri* or those accompanying the disease exanthema. In that disease the region of the oil vesicles in the rind is impregnated with gummy substances of the nature of pentosans, which, as these writers

³ Reference is made by number (italic) to Literature Cited, p. 220.

suggest, may absorb water excessively when the water supply is plentiful and so cause rupture through abnormal swelling.

Boussingault in 1873 (2) demonstrated the ability of fruits of cherry, plum, pear, grape, and other horticultural forms to absorb water through the skin when submerged for a long period. Hartman and Bullis (16) reported that cracking of sweet cherries occurred in the Willamette Valley, Oreg., as a result of excessive water absorption by the fruit, either directly through the skin in wet weather or by way of the root system and vessels.

In extensive experiments with different periods and amounts of irrigation, Verner and Blodgett (29) were unable to observe any relationship between soil moisture and cracking in the three varieties of sweet cherries with which they experimented, although they tested extremes of soil moisture far beyond those usually found in irrigated orchards. Sawada (21), who experimented in Japan on the splitting of sweet cherries, likewise concluded that extremes of soil moisture played no direct part in producing the injury. He flooded the soil about the root system of a potted tree bearing mature fruit, but this drastic treatment failed to produce cracking.

Sawada was able to prevent cracking of sweet cherries on the tree by enclosing fruits in paraffined paper. By excluding rain by means of waterproof tarpaulins, Verner and Blodgett (29) succeeded in preventing the cracking of ripening fruit on large branches of sweet cherry trees in rainy periods, when severe cracking occurred on the exposed parts of the same trees. They concluded that cracking in this fruit was due mainly to direct absorption of rain water through the fruit skin. When fruits were held for a time under water, high rates of water absorption and pronounced cracking were both found to be concomitant with high concentrations of solutes in the expressed fruit juice, this concentration being regarded as a rough measure of the osmotic value of the cell sap.

Howard⁴ states that he has observed splitting of sweet cherries in orchards at Mountain View, Calif., near San Francisco Bay, in periods of heavy fog without rain and without change in soil-moisture content through irrigation, and he suggests that this phenomenon may have been due to a sudden increase in the rate of water supply to the fruits, which might result from a decrease in the rate of water loss from the leaves. He says that root pressure might, under such circumstances, promote cracking by causing water to be forced into the fruit tissues from the vessels. Rixford (20) states that figs have been observed to split under conditions of high atmospheric humidity without rain or irrigation.

In the varieties of apples known as Cox Orange and Dunn, Campbell (7) and Goodwin (13) describe a russetting and cracking that led to great losses in the Nelson district of New Zealand. The latter writer, who describes the skin breaks of these apples as occurring only in conjunction with russetting or with lesions produced by the conothecium disease, considers the disorder as dependent upon general debility of the tree.

Sorauer (23) discusses the rupture of fleshy plant parts in general, considering the causal relations to be much alike in the many different forms of this disorder that he mentions, including fruit cracking in

⁴ HOWARD, W. L. Correspondence. 1932.

cherry, plum, and grape, bursting of carrots and beets, and splitting of stems in kohlrabi, rape, bean, and potato. He says (23, p. 322): "All these phenomena have one characteristic in common—that they are initiated only when, after a considerable period of normal development, or still more after a previous dry period, an unusual supply of water is given suddenly."

According to Graebner (14), periods of drought result in the development of strengthening tissues, which usually appear first in the xylem and phloem. Mechanically strengthened cells have, as a rule, lost their ability to divide and most of their capacity to enlarge. If the water supply is greatly increased after a dry period, in the course of which the growth ability of some cells has been lost or has been greatly reduced, then the meristematic groups quickly resume growth, but corresponding growth cannot occur in the strengthened cells. Resultant differences in growth rates between contiguous mechanical and meristematic tissues may thus lead to excessive tensions, and rending of the mechanical tissue may ensue.

In a recent study by Frazier⁵ it was found that tomatoes cracked most severely after heavy irrigation at the end of a prolonged dry period. Cracking was less severe in plots with frequent irrigation, which prevented excessive drying of the soil, and it was least severe in plots where the soil-moisture content remained low throughout the growing season. Shaded fruits cracked much less than those exposed to the sun.

Tracy (25) states that cracking of tomatoes may be prevented by enclosing the fruits when half grown in paper bags; but he points out that this treatment slightly impairs the flavor of the ripe fruit.

METHODS OF EXPERIMENTATION

The conditions that were studied in relation to cracking of apples may be divided into (1) external and (2) internal. The external conditions included those of weather (air temperature, air humidity, precipitation, evaporativity) and those of the soil, especially soil-moisture content. The internal conditions included rate of fruit enlargement, abnormalities of the peripheral region of the fruit, and freezing-point depression of the cortical tissue.

WEATHER DATA

Continuous records of temperature and relative humidity of the air were obtained throughout the growing season by means of a hygrothermograph installed in a standard United States Weather Bureau shelter located about 400 feet from the experimental block of trees. Temperatures and percentages of relative humidity, read directly from the automatic records at 2-hour intervals, were tabulated and employed as basic data. Temperature readings were used without modification, but corresponding readings of relative humidity and temperature were combined to give values of the vapor-pressure deficit.

Direct indices of the evaporating power of the air and of total sunshine intensity were obtained by means of Livingston porous porcelain atmometers (18, 19), both white and black spheres being used. The black sphere was of the new type, made of black porous porcelain.

⁵ FRAZIER, W. A. A STUDY OF SOME FACTORS ASSOCIATED WITH THE OCCURRENCE OF CRACKS IN THE TOMATO FRUIT, 1933. (Ph. D. diss. Univ. Md.)

For 5 weeks in 1933, when the apples were thought most likely to crack, the black sphere was mounted on one end of an upright U tube of small bore (4 mm), the other end of which was joined to a vertical glass cylinder 3 cm in diameter and 24 cm high, open at the top, which served as a reservoir for supplying the atmometer with distilled water. Within this cylinder was a light, cylindrical glass float filled with air and sealed. To this float was attached a small vertical wire bearing a pen so adjusted that, as the float gradually descended in response to withdrawal of water from the reservoir, a continuous record was traced on a paper sheet borne by an upright, clock-driven drum. The slope of the tracing was thus a measure of the rate at which water evaporated from the standard black sphere.

Depth of rainfall, measured by a standard United States Weather Bureau rain gage, was recorded at intervals not exceeding 24 hours.

PROTECTION OF FRUIT FROM WETTING BY RAIN

Because water absorption through the fruit skin in periods when the latter is covered with a film of liquid water—as during and following times of precipitation—might conceivably be among the conditions that lead to cracking, some apples were covered to protect them from wetting by rain. This was accomplished by spraying with mineral oil emulsions or fish-oil soap, by enclosing apples in paper or cellophane bags, and by covering large branches of apples with waterproof tarpaulins in times of rain.

SOIL TREATMENT AND MEASUREMENT OF SOIL MOISTURE

The soil in the experimental block of trees is a clay loam of good fertility belonging to the Hagerstown series (5). Throughout the periods of observation on cracking there was always a moderately good stand of volunteer growth of weeds and grasses, which was undisturbed at these times.

In a region like West Virginia, which is subject to frequent and sometimes heavy summer rains, the experimental establishment of very marked differences in soil-moisture conditions in the field is very difficult, but an attempt was made to establish and maintain such soil-moisture differences among the experimental trees as were feasible. Throughout the periods when the likelihood of cracking was greatest, these differences were about as large as are ever found in orchards of this region. Special treatments with regard to soil moisture were as follows: (1) In 1933 the root system of one tree was deprived of the benefits of rains by diverting rain water from the root zone by means of waterproof tarpaulins supported on a wooden framework so arranged as to cover the soil area within and slightly beyond the spread of the branches, the intercepted water being discharged outside of that area. A marginal trench about 12 inches back from the outer edge of the tarpaulin covering served to prevent water seepage into the covered area. This trench was about 30 inches deep, extending at least 12 inches below the lowest roots intercepted, which were all cut. On October 6, when the fruit was nearly mature and when the moisture content of the upper 12 inches of soil under the tarpaulins had been reduced nearly to the wilting point, a flood irrigation equivalent to 4 inches of rainfall was given. (2) Early in the summer of 1933 several trees were mulched with

straw, the mulched area being somewhat broader than the spread of the branches. The covering, which was 4 to 6 inches deep after settling, was calculated to greatly retard water loss.

Soil samples were taken in 1933 at intervals of from 2 to 4 days between August 12 and October 7, embracing a period well in advance of, and including, that in which the most severe cracking is usually observed. At each time of sampling a cylinder of soil was taken, by means of a California soil tube (27), from the upper 6 inches and from the second 6 inches of soil. Samples were secured from three or more locations among the experimental trees, and the moisture percentages, calculated on the dry-weight basis, were averaged to give a single reading, which is taken to represent the soil of the experimental block as a whole.

FREEZING-POINT DETERMINATIONS

Freezing-point depressions of samples of apple tissue were ascertained from time to time by means of a Beckmann thermometer, a special tissue-cup method described by Verner (28) being used.

FRUITS UNDER WATER

Direct absorption of water through the fruit skin was studied for detached apples by sealing each stem and calyx opening with paraffin, weighing the fruits thus sealed, and then reweighing them after they had been kept under tap water for a time. Branches with fruits still attached were bent down and held with the fruit under water in 50-gallon barrels. By means of a steel tape graduated in millimeters the largest horizontal circumference of each apple was measured before and after this water treatment, and these measurements were compared with corresponding measurements of untreated fruits on adjacent branches.

RESULTS AND DISCUSSION

GENERAL OBSERVATIONS

VARIABILITY IN EXTENT OF CRACKING

Field observations on apples have revealed marked differences in the incidence of cracking in the same orchard in different seasons, in different orchards in the same season, and in the same season among individual trees of the same orchard and branches of the same tree. For York Imperial and Stayman Winesap the extent of cracking in different orchards varied in 1932 from less than 5 to over 60 percent. At the experimental orchard near Kearneysville the percentage of fruit that cracked per Stayman Winesap tree varied from 33 to 65, the extremes appearing in adjacent trees. Two different branches of the same Stayman Winesap tree showed, respectively, 31 and 70 percent of cracking. In several cases one fruit of a spur was badly cracked while a second fruit of the same spur, with almost the same exposure and with the same outward appearance, was unaffected. Thus, while weather conditions exert a major influence in the cracking of apples, it is apparent that physiological conditions within the tree or fruit, not directly related to current weather features, are also influential.

NATURE AND RATE OF CRACK FORMATION

In periods when weather conditions were such as to warrant the prediction that cracking would soon occur, many fruits were examined with a hand lens at frequent intervals, and it was thus possible to detect incipient cracks when they were much less than a millimeter long and to observe the regions in which they occurred and the manner in which they enlarged and multiplied. It was evident that cracks generally appeared first in restricted areas, especially in areas of russeting, sunburn, or high skin coloration. It appeared as though the peripheral tissues were exceptionally weak in such regions or the tissue strains were more pronounced there than elsewhere. Localization was usually very definite, and it was often possible to detect a dozen or more minute breaks within an area of a square inch. Enlargement of cracks sometimes continued for several days, especially if evaporation rates were low.

Many incipient cracks apparently originated at hypertrophied lenticels of the cracking area (fig. 2). If the cracks remained small until after the weather became drier, cork was formed in the minute fissures and a russeted area might thus result. The russeting here considered may therefore have resulted, at least in part, from lenticel hypertrophy, incipient cracking, and subsequent cork formation.

Several writers have concluded that lenticel hypertrophy may be caused or promoted by greatly retarded transpiration from the plant, accompanied by a plentiful water supply to the regions of hypertrophy. Thus the formation of the corky or mealy excrescences that mark lenticel hypertrophy on young stems and branches of such trees as cherry and elder are attributed by Sorauer (23) to temporary retardation of foliar transpiration in periods of high atmospheric humidity. Schilberszky (22) concluded that hypertrophy of lenticels in apple fruits is related to an excessive water supply in the soil. Devaux (10) considered lenticel formation to be intimately connected with excessive moisture supply in underlying tissues, and Swingle (24) found that lenticel hypertrophy in stem cuttings of willow was directly correlated with their water supply under conditions of negligible evaporation. The proliferation that constitutes lenticel hypertrophy may decrease the extensibility of the neighboring peripheral cell layers and lower their mechanical resistance to being torn apart; and if that be true lenticels might be expected to mark the weakest points, at which rupture should begin whenever peripheral tissue strains become sufficiently excessive.

As to the formation of large cracks, numerous observations showed that it usually required at least several hours for these to develop. Thus, in one instance, the development of a crack 11 mm in length and about 1 mm in maximal depth required 3 hours, counting from its first clearly visible appearance as a mere slit about a millimeter long. Some cracks developed more rapidly; one attained a length of about 60 mm in 6 hours after its inception. A representative sample of the larger cracks showed successive lengths of 11, 18, 22, and 43 mm at 48-hour intervals.

RELATION OF NATURAL ENVIRONMENTAL CONDITIONS TO CRACKING

EXPLANATION OF GRAPHS

Data on natural fluctuations in soil-moisture content, rainfall, atmometric evaporation, and air humidity in relation to cracking of apples on the experimental trees in 1933 are summarized in figure 3.

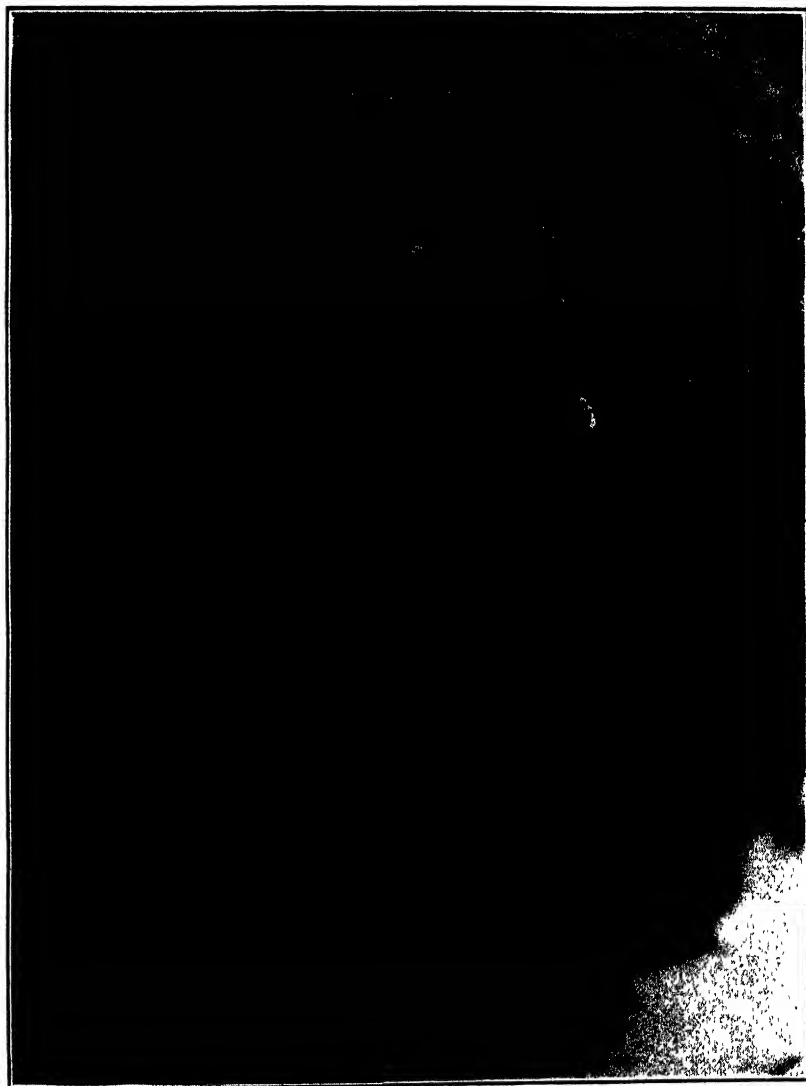


FIGURE 2.—Hypertrophied lenticels, cracks, and russet development in a Stayman Winesap apple. $\times 3$.

The smallest subdivision on the abscissa represents a 2-hour interval. The 5-week period considered (Sept. 3 to Oct. 8) covers the latter part of the period of fruit enlargement. The ordinate scale represents the index values of the respective environmental features.

The two upper graphs present the general march of soil-moisture percentage on the dry-weight basis. In the arbitrary scale of ordinates at the left, unity represents a moisture percentage of 4, which is the scale factor for these two soil-moisture graphs.

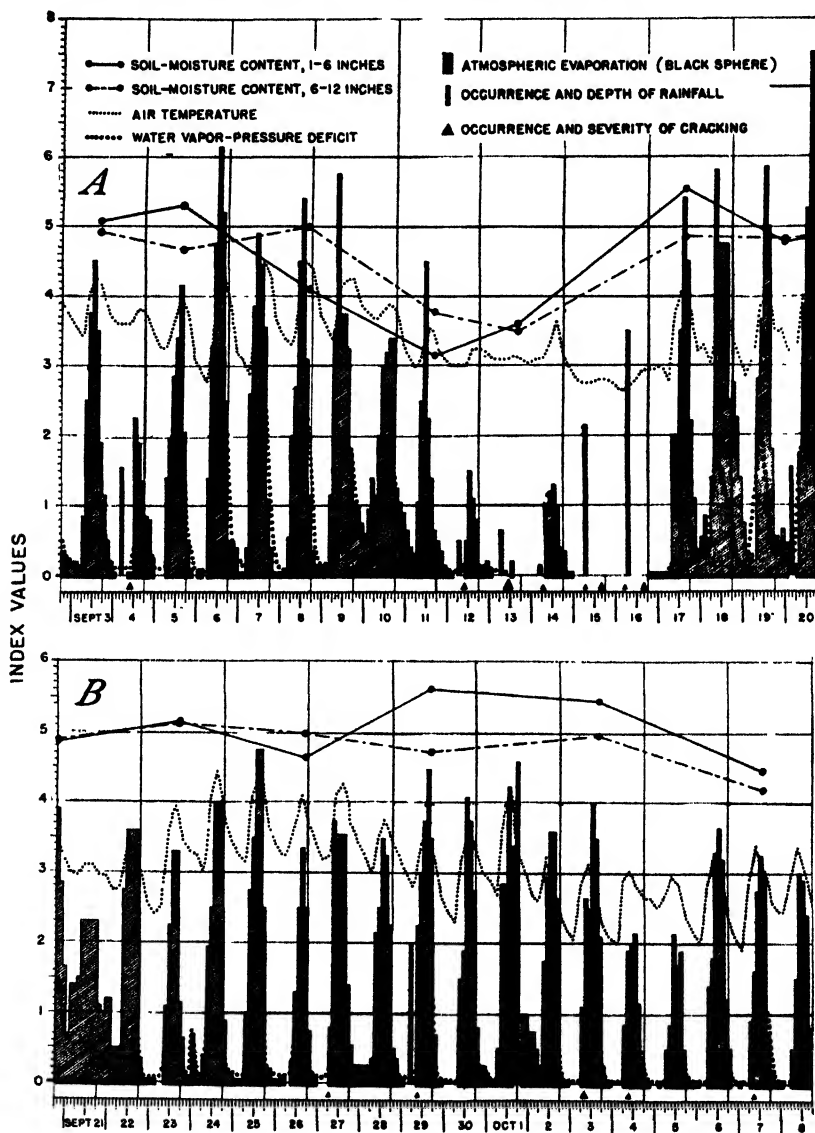


FIGURE 3.—Relation of cracking in Stayman Winesap apples to fluctuations in environmental conditions Sept. 3 to Oct. 8, 1933. See text for complete explanation.

The march of air temperature is shown by the narrow dotted line, which is derived from the automatic tracing of the thermograph. The scale factor for air temperature is 20; that is, unity on the general ordinate scale at the left represents 20° F.

The wider dotted line shows the march of the water-saturation deficit of the air. This deficit is taken to represent the current evaporating power of the air with the wind and radiation factors removed. For this feature unity on the ordinate scale corresponds to a water-vapor pressure deficit of 8 mm of a barometric mercury column.

The occurrence of measurable rainfall is indicated by vertical black bars. The height of any bar shows the total depth of rain accumulated in the 24-hour period ending at 6 p. m. on the day referred to. Duration of rainfall was not recorded; therefore only a single bar is shown for each day with measurable precipitation, and all these bars have the same width, which is arbitrary. The scale factor for rainfall is 0.2 inch or approximately 0.5 cm.

Fluctuations in the rate of evaporation from the standardized black porous-porcelain atmometer sphere, exposed in the open, are shown by the hachured stepgraph of figure 3. This graph is plotted for every 2-hour period, as though it consisted of vertical bars, each 2 hours in width. The height of each bar or step represents the total accumulated water loss for its period. The scale factor for evaporation is 2 ml per 2-hour period; that is, unity on the general ordinate scale corresponds to water loss from the instrument at a mean rate of 1 ml per hour for the corresponding 2-hour period. It is at once obvious that the intensity of this feature fluctuated greatly and that there were occasional periods without measurable evaporation. For such periods this graph naturally coincides with the base line. The horizontal extent of each hachured block has been indicated by broadening the base line, and intervals without measurable water loss from the atmometer are clearly shown by the absence of the broad base line. Periods without evaporation, or periods of very slow evaporation, are of special interest, as will later appear.

Observations on cracking of the fruit were made at intervals of 2 or 3 hours in the daytime, but no observations of this sort were made at night. For each observation cracking was recorded only for the number of fruits that had been noted as uncracked at the last preceding observation. Consequently the total number of observed fruits used as a basis for these percentages became smaller as the season advanced. Naturally, the most susceptible fruits were the first to crack when external conditions favored this injury, and later observations on occurrence and degree of cracking were confined to fruits that had not already been recorded as cracked. In the present connection all cracks were considered as equal, and no attention is here given to additional cracking of fruit previously recorded as showing cracks.

Because of these considerations the percentage values derived from the observational counts should be adequately weighted or re-evaluated before being employed quantitatively in this part of the study, but no satisfactory way of so modifying them has been found. Therefore the cracking record presented in figure 3 is in very general terms as (1) slight, (2) moderate, and (3) severe; representing, respectively, about the following values: (1) from 1 to 5 percent, (2) from 6 to 15 percent, and (3) more than 15 percent. These three relative degrees of cracking are indicated, respectively, by small, medium-sized, and large black triangles placed just beneath the base line; the abscissal position of each triangle shows when the corresponding outbreak of cracking was observed.

RELATION OF AIR TEMPERATURE AND SOIL MOISTURE TO CRACKING

There appears to be no correlation at all between air-temperature fluctuation and the occurrence of cracking, so far as may be judged by a study of the graphs of figure 3.

The fluctuations of soil-moisture content shown in figure 3 exhibit no relation to cracking. These fluctuations were relatively slight, and it is safe to suppose that the soil-moisture content of the first 6-inch and the second 6-inch depth was, in the 5-week period considered, never low enough to approach closely the value of the wilting coefficient of this soil. The wilting coefficient of the soil of the experiment orchard at Kearneysville may be estimated as about 10 percent on the gravimetric basis and according to Veihmeyer's (26) interpretation of this apparently critical value. The depth to which the present records refer (0 to 12 inches) is estimated to have included the region occupied by more than three-fifths of the roots of these trees.

Veihmeyer has shown (26) that the rate of water utilization by prune and peach trees with which he experimented in southern California was independent of the amount of moisture present in the soil as long as this amount was above the wilting coefficient. He found that the rate at which water was removed from the soil by a tree appeared to be determined primarily by the evaporating power of the air and the magnitude of the total leaf surface of the tree. If this is generally true of fruit trees it is hardly to be expected that changes in soil moisture within the limits usually met with in nonirrigated orchards—that is, above the wilting point and below field saturation—should affect changes in the water balance of the tree sufficient to produce cracking.

RELATION OF RAINFALL TO CRACKING

Since soil-moisture content shows no apparent relation to cracking, wetting of the soil by rain is not to be regarded as among the influences that promoted this injury. If the occurrence of rain really promoted cracking in this 5-week period its influence must have been exerted in some other manner than through an increase in the supply of water to the tree roots. During rainy periods the foliage and the apples themselves were, of course, more or less completely covered with water, and it is conceivable that direct absorption of water through leaf epidermis and fruit skin may have been directly or indirectly influential in promoting the tissue strains that lead to cracking.

An indirect way in which the occurrence of a rainy period following a drier one might promote cracking is through sudden lowering of the transpiration rate, for transpiration must be practically prevented as long as the transpiring surfaces are covered with water. But in periods of little or no evaporation, transpiration must be very slow whether or not rain occurs.

However these interdependent variables, rain and evaporativity, may operate in general, a study of the graphs in figure 3 makes it clear that, although the outbreaks of cracking usually were preceded immediately by rain, this was not always so. Thus in several instances (on Sept. 27 and Oct. 3, 4, and 7) cracking was observed when there had been no recorded rain for from 36 hours to several days, and in other instances (Sept. 20 and Oct. 1) cracking failed to occur in spite of heavy rains.

RELATION OF ATMOMETRIC EVAPORATION TO CRACKING

Figure 3 shows, for the 5-week period represented, a very clear and consistent relation between occurrence of cracking and occurrence of very low rates of evaporation from the black atmometer sphere. Cracking was never observed except after a period of slow evaporation, and nearly every period of 6 hours or more without recorded evaporation was accompanied or immediately followed by cracking. On October 7 cracking followed two 4-hour periods without evaporation, the two periods being separated by a 2-hour interval with very low rate. On the other days when cracking occurred it was preceded in every instance by at least 6 hours without measurable water loss from the atmometer. As has been mentioned, although prolonged periods without atmometric evaporation were, in a number of instances, concomitant with periods of rainfall, there were also periods of cracking that obviously were not related at all to the occurrence of rain. Examination of figure 3 shows that these periods of cracking without antecedent rain (Sept. 27 and Oct. 3, 4, and 7) in every case were immediately preceded by from 6 to 8 hours of little or no measurable atmometric evaporation. Conversely, as has just been pointed out, on October 1 no cracking was observed during or following a rain of 0.92 inch, when the evaporation rate was zero for only 4 hours, then rose abruptly; nor was there any cracking following the lighter rain of September 20, when, also, the period of slow evaporation was short. The nonoccurrence of cracking in the prolonged periods of low evaporation on September 5 and September 30 may, perhaps, be accounted for partially or wholly by the fact that in each case there had been considerable cracking on the preceding day; presumably the apples currently most susceptible to this injury had already cracked.

RELATION OF WATER VAPOR-PRESSURE DEFICIT OF THE AIR TO CRACKING

As would be expected, the graphs of vapor-pressure deficit have almost exactly the same form as the corresponding ones of atmometric evaporation. The period from September 13 to 16 (fig. 3) includes two intervals for which this deficit has zero value and both of these are without measurable atmometric evaporation. Also, both show records of severe cracking. The remaining intervals without measurable evaporation from the black sphere, as these are represented in figure 3, show very low deficit values of 1 mm or less. All the outbreaks of cracking recorded in this figure were preceded by 4 or more hours with vapor-pressure deficit below 1.6 mm.

A fairly close relationship was found to exist between relative humidity, or relative-humidity deficit, and cracking. The most extensive cracking in any 24-hour period in 1933 took place on August 24 in a 10-hour period when relative humidity was recorded as 100 percent. This period followed a rainfall of 4.25 inches. In 1932 approximately 40 percent of the crop of Stayman Winesap apples in the experimental orchard were damaged by cracking in a 38-hour period on October 17 and 18, when the recorded relative humidity at no time was below 99 percent. The total rainfall for these 2 days was 2.07 inches, but the soil had a high water content before the rain began. A heavier rain on October 5 and 6, totaling 2.85 inches

but accompanied by humidity for the most part well below 90 percent, caused no cracking. Similar instances of apparent association of cracking with prolonged high air humidity, either with or without rainfall, were observed many times in 1932 and 1933 and in several varieties of apples, including Stayman Winesap, York Imperial, Northern Spy, Wealthy, and Yellow Transparent. At no time during these 2 years was cracking observed except during periods of 8 hours or more when relative humidity was above 90 percent.

ORCHARD EXPERIMENTS WITH MODIFICATION OF NATURAL ENVIRONMENTAL CONDITIONS

MODIFICATION OF SOIL-MOISTURE CONTENT

In an attempt to obtain somewhat greater extremes in soil-moisture content than ordinarily occur in the region in which these experiments were conducted, the soil over the root system of one tree was protected from rain for several weeks by means of waterproof tarpaulins, as has been noted. This shelter was erected on August 31, 1933, when the average soil-moisture percentage for the upper 12 inches in the experimental block was 18.1. It was removed on October 6, when the 12-inch index for the sheltered soil had decreased to 12.4 percent and that for the rest of the block was 17.4 percent. Immediately after the removal of the tarpaulins the soil area that had been covered was given a flood irrigation equivalent to 4 inches of rainfall. About 10 hours later the 0- to 12-inch soil-moisture content for this area was found to have increased to 20 percent. This sudden increase in soil moisture failed to produce any cracking. Similar irrigation of one tree in 1932, without previous use of tarpaulins but following prolonged dry weather, had also failed to produce cracking. A heavy straw mulch had no apparent influence on cracking, although such a soil cover retards water loss from the soil while it permits free penetration of rain water.

Between August 31 and October 6, 1933, there was cracking of the fruit on the tree with the tarpaulin-sheltered root system when cracking was common among other trees of the experimental block. Since the soil about the roots of this tree was protected from being wetted by rain throughout that period, it is clear that wetting of the soil was not required to produce cracking. Whether or not a sudden and sufficiently pronounced rise in soil-moisture content following a period of very dry soil may lead to cracking of apples under certain conditions is, of course, still an open question; but the present study furnishes no clear evidence that wetting of the soil was causally related to the cracking here recorded. Under the natural and the artificially modified soil conditions of this study, it appears that very slow atmospheric evaporation was probably the immediate external cause of cracking.

FRUITS PROTECTED FROM WETTING BY RAIN

With the possibility in mind that direct water absorption through the apple skin in a period of rain may sometimes be a contributing cause of cracking, the following experiment was carried out. A large

branch, bearing about 50 apples, was covered with a waterproof tarpaulin when rain occurred, the cover being removed when rain had ceased and the rest of the foliage was no longer wet. In a period of no atmometric evaporation, which was accompanied by rain, three of these protected apples cracked in spite of the fact that their skins were superficially dry. Although the number of apples involved in this test was not sufficient to furnish general evidence as to whether the prevention of wetting reduced the percentage of cracking, the result clearly supports the evidence already discussed that wetting of the fruit surface by rain is not, at least in general, necessary to cause cracking. The presence of rain water on the fruit may, however, favor cracking when other influences also are favorable to it.

ATTACHED BRANCH UNDER WATER

On September 30, 1932, a large Stayman Winesap branch bearing fruit without any cracks was bent down into a barrel of water so that all the apples and most of the leaves and small branches were submerged. This experiment continued for 4 days, and in that time severe cracking occurred. In the 4-day interval there were no periods of very low evaporation rates and no change of soil-moisture content other than the usual slow decrease due to ordinary evaporation and root absorption. At the same time no cracking of normally exposed fruits was observed, either on the same tree or on others. Measurements of maximal horizontal circumferences of the submerged apples, and of those on an adjacent untreated branch of the same tree, showed that in the 4-day period the mean rate of enlargement of the submerged fruits was much greater than that of the exposed ones. Details of this experiment are presented in table 1.

The fruits of both groups were originally of about the same size. The maximal enlargement percentage for submerged fruits (2.52) is almost double that for exposed fruits (1.34). The average enlargement percentages are 1.47 (submerged fruits) and 0.93 (exposed fruits), the former being about 1.58 times the latter; that is, the apples on the branch under water increased their maximal horizontal circumferences, on an average, about 58 percent more rapidly than did the apples on the exposed branch.

Twenty of the thirty-four submerged fruits cracked in the 4-day interval considered, but there was no cracking among the exposed fruits. The submerged fruits may be classified in three groups, according to their rates of enlargement, nos. 1 to 11 being considered as enlarging slowly, nos. 12 to 22 as enlarging at an intermediate rate, and nos. 23 to 34 as enlarging rapidly. The average length of cracks in the slowly enlarging group was 0.75 cm (2 out of 11 apples cracked), in the median group 6.0 cm (7 out of 11 apples cracked), and in the rapidly enlarging group 7.6 cm (11 out of 12 apples cracked).

TABLE 1.—*Enlargement rates of Stayman Winesap apples still attached to tree, some on branch submerged in water and others normally exposed on adjacent branch of same tree, together with records of cracking for submerged fruits, September 30 to October 4, 1932*

[No cracking of exposed fruits occurred. Data are arranged in ascending order of enlargement rates in each group]

Apple no.	On submerged branch			On adjacent branch	
	Sept. 30, original circum- ference	Oct. 4		Sept. 30, original circum- ference	Oct. 4, increase in circum- ference
		Increase in circum- ference	Total length of all cracks		
	Centimeters	Percent	Centimeters	Centimeters	Percent
1.....	22.95	0.22	(¹)	22.95	0.65
2.....	22.05	.45	(¹)	22.70	.60
3.....	24.05	.63	(¹)	22.15	.68
4.....	22.95	.65	1.1	21.30	.70
5.....	22.70	.66	(¹)	21.55	.70
6.....	19.65	.76	7.1	19.65	.76
7.....	23.40	.85	(¹)	24.30	.82
8.....	23.10	.86	(¹)	24.15	.83
9.....	22.30	.90	(¹)	23.60	.84
10.....	22.10	.90	(¹)	23.40	.85
11.....	22.75	.90	(¹)	22.20	.90
12.....	24.35	1.23	11.8	21.70	.92
13.....	19.55	1.28	(¹)	21.12	.94
14.....	23.10	1.30	(¹)	20.50	.97
15.....	23.20	1.30	1.1	20.00	1.00
16.....	23.00	1.30	1.4	24.65	1.02
17.....	21.65	1.39	(¹)	24.05	1.04
18.....	22.50	1.51	5.7	23.90	1.04
19.....	22.50	1.51	17.6	23.20	1.08
20.....	21.80	1.60	(¹)	23.00	1.09
21.....	20.70	1.69	14.8	22.75	1.10
22.....	23.50	1.70	14.5	22.60	1.10
23.....	21.50	1.86	(¹)	22.85	1.10
24.....	24.05	1.87	1.5	22.55	1.11
25.....	21.10	1.90	6.9	21.35	1.17
26.....	23.20	1.94	12.0	21.20	1.18
27.....	21.90	2.05	10.0	23.70	1.26
28.....	20.55	2.20	9.9	23.70	1.26
29.....	24.50	2.26	3.2	23.35	1.29
30.....	22.10	2.26	9.6	23.05	1.30
31.....	21.90	2.28	6.3	22.90	1.31
32.....	21.65	2.31	5.8	22.30	1.34
33.....	21.60	2.33	4.9		
34.....	23.85	2.52	21.5		
Average.....	22.40	1.47	4.9	22.57	.93

¹ No cracking.

While the water treatment seems, in general, to have increased the incidence of cracking, especially when it also increased the rate of fruit enlargement, the severity of cracking was apparently influenced by conditions not taken into quantitative account, presumably by variable internal characteristics of the fruits at the time of the experiment. The occurrence of cracking seems to be dependent not so much upon the rapid enlargement of the fruit as a whole as upon extra rapid enlargement of a restricted region of the tissues. The following chance observation made on September 16, 1933, may bear on this suggestion. In an apple with more than a third of its volume involved in a soft rot extending from the surface to the core and so advanced that the diseased portion was reduced to a mass of soft pulp, severe cracking had taken place on the uninfected part. This cracking must have been entirely independent of enlargement of the fruit as a whole.

LEAF INJURY AND DEFOLIATION

In a large block of Stayman Winesap trees near Shenandoah Junction, about 3 miles east of the Kearneysville orchard, which was used for most of this study, the crop of 1933 showed very little cracking (less than 0.5 percent), while 40 percent of the same year's crop at the Kearneysville orchard was cracked. The trees of these two orchards were of the same age (14 years), both orchards were on the same type of soil, and weather conditions must have been virtually the same for both. The two orchards were noticeably different in that the Kearneysville trees had been partially defoliated or their foliage had been somewhat injured by spray materials, whereas the foliage in the other orchard was unusually dense and healthy. More of the fruits at Kearneysville were partially russeted, probably due largely to spray injury, and more of them were sunburned or highly colored, due to greater exposure to sunlight. All of these conditions, i. e., russetting, sunburn, and high coloration appear to be associated with increased cracking. Similar relationships between foliage conditions and cracking were seen among individual trees and branches in the experimental block at Kearneysville, where the highest percentages of cracking were associated with the highest percentages of leaf injury or with the sparsest foliage.

Figure 4, *A* and *B*, shows the general appearance of densely foliated branches with little cracking as compared with sparsely foliated branches with severe cracking. Data of a more quantitative nature bearing on this relation between foliage density and severity of cracking were secured by comparing three representative branches of about the same size but showing different degrees of cracking with respect to the total fresh weight of the leaves. One branch (*A*, at Shenandoah Junction) showed no cracking and the other two (nos. 2-22 and 4-9, at Kearneysville) showed, respectively, slight and severe cracking. The apples on all three of these branches were virtually free of russet and other visible skin injuries. Table 2 presents these data.

TABLE 2.—Comparative data on number and diameter of fruit and fresh weight of leaves as related to the occurrence of cracking in apples

Branch no.	Apples on branch	Average diameter of apples	Total fresh weight of leaves	Degree crackin
	Number	Inches	Grams	
22	108	2.75	1,710	Nona.
72	72	2.25	1,518	Slight.
9	76	2.75	828	Severe.

ATTACHED APPLES ENCLOSED IN BROWN-PAPER BAGS

In the autumn of 1932 about 200 Stayman Winesap apples on three experimental trees were bagged and these were observed from time to time, observations being made, at the same times, on a similar number of comparable fruits, previously tagged, on these same trees. Cracking of the unbagged fruits severe enough to furnish a useful measure of the effects of bagging did not occur until the bagged apples had been enclosed for 3 to 4 weeks—time enough for pronounced morphological and physiological alterations to take place. Therefore the bagged

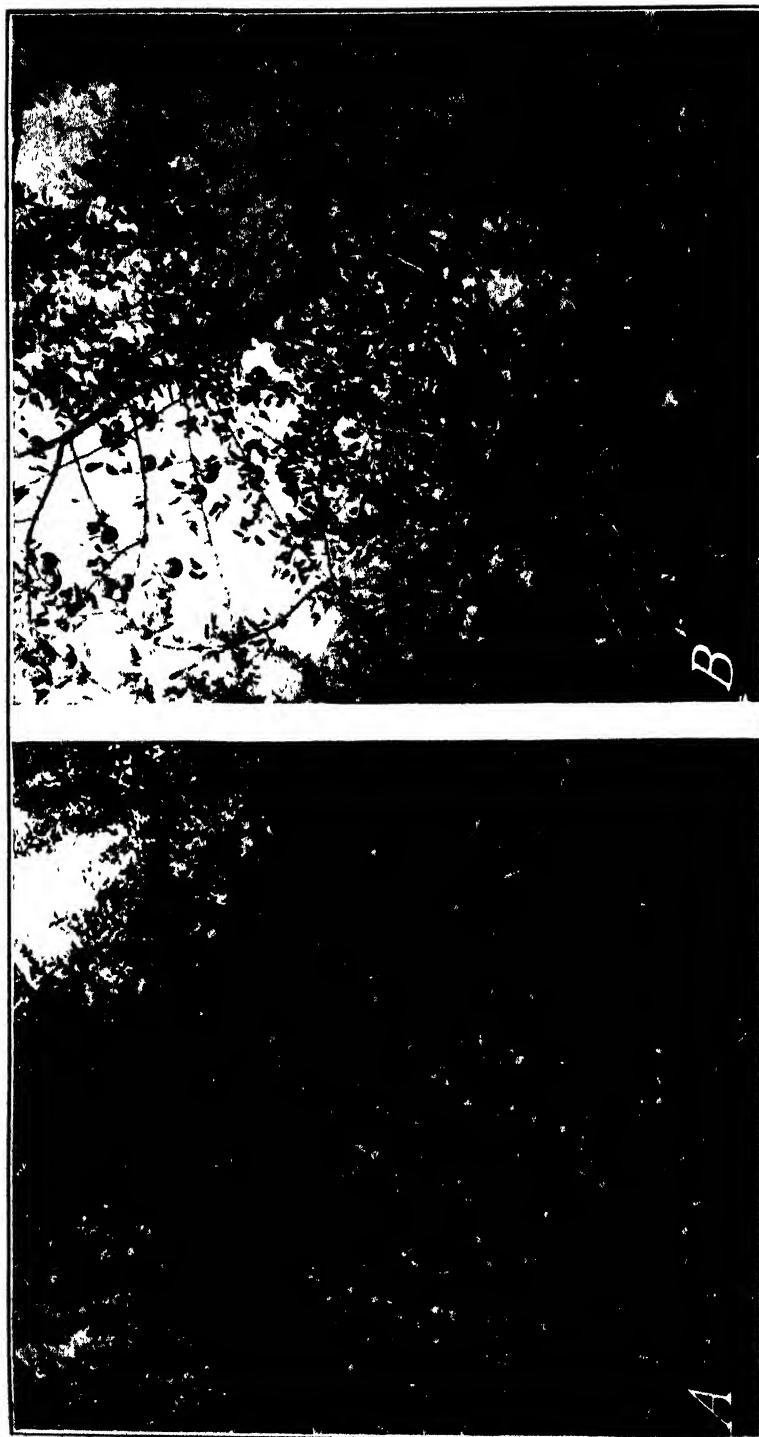


FIGURE 4.—A, Part of a Stayman Winesap tree with dense foliage and little cracking, near Shenandoah Junction; B, part of a Stayman Winesap tree with sparse foliage and severe cracking, near Kearneysville, W. Va.

apples probably differed markedly from the controls long before the latter showed much cracking. When about 30 to 50 percent of the controls had cracked, only about 2 to 6 percent of the protected apples had cracked. This is shown by records taken on October 20, presented in table 3.

TABLE 3.—*Cracking of bagged and unbagged Stayman Winesap apples*

Tree no.	Bagged apples			Unbagged apples	
	Apples	Days bagged	Cracked	Apples	Cracked
	Number	Number	Percent	Number	Percent
3-12	110	30	6.4	90	54.4
4-11	50	27	6.0	50	30.0
4-12	50	20	2.0	50	30.0

By harvest time the enclosed apples had developed the solid yellow ground color characteristic of this variety at full maturity, but they were devoid of any trace of red overcolor. It is reasonable to suppose that protection for many weeks from the direct rays of the sun might so influence either the osmotic values or the structural characteristics of the apple as to make it less inclined to crack. The effects may, therefore, have been due to prolonged shading, just as dense foliage seems to have reduced the tendency toward cracking.

OIL-EMULSION SPRAYS

In 1932 extensive tests were conducted with different concentrations, and with different times of application, of fish-oil and mineral-oil sprays, on the supposition that the oily films left on the fruits might retard or prevent absorption of water through the skin and so reduce cracking. No effects upon cracking were observed, however. When sprayed and unsprayed apples were subsequently tested under water it was found that the sprayed fruits although distinctly oily to the touch, absorbed water about as rapidly as did the untreated ones. Apparently the oil sprays used had failed to reduce the permeability of the skin to water.

FRUITS REMOVED FROM THE TREE AND SUBMERGED IN WATER

The evidence thus far presented leads to the conclusion that wetting of the soil by rain or irrigation was not a considerable influence in promoting cracking in the experimental orchard. The other possible influence of rain (i. e., the maintenance for a time of a more or less complete liquid water layer over the fruit and foliar surfaces) is not shown by figure 3 to have been influential in producing cracking; but when an attached branch bearing fruit and leaves was kept under water for 4 days this treatment was very effective in producing cracking, especially in fruits in which the water treatment accelerated enlargement. The experiment with the submerged branch was supplemented by some additional experiments with apples removed from the tree and kept under water.

After the cut end of the stem and the calyx opening had been sealed with paraffin each apple was weighed and all were placed in water, where they were held for 2 days. They were finally wiped and reweighed, the weight gain of each was computed, and the degree of cracking shown by each was recorded as the total length of all its cracks. Data from this experiment with mature apples removed from the trees are shown in table 4.

TABLE 4.—*Weight increase and cracking in mature Stayman Winesap apples kept under water for 2 days, Oct. 24 to 26, 1931*

[Apples are arranged in the ascending order of their weight gains]

Apple no.	Original weight	Gain in weight	Total length of all cracks	Apple no.	Original weight	Gain in weight	Total length of all cracks
	Grams	Percent	Centimeters		Grams	Percent	Centimeters
1.....	207	0	(1)	11.....	255	2.35	3.75
2.....	222	.90	(1)	12.....	286	2.45	(1)
3.....	195	1.03	(1)	13.....	244	2.46	4.25
4.....	243	1.24	(1)	14.....	219	2.74	14.75
5.....	158	1.26	2.75	15.....	166	3.01	4.50
6.....	241	1.66	4.25	16.....	195	3.08	4.25
7.....	172	1.75	1.00	17.....	246	3.25	11.25
8.....	172	1.75	1.75	18.....	237	3.38	(1)
9.....	198	2.02	2.25				
10.....	220	2.27	1.25	Average.....	215	2.32	3.11

¹ No cracking.

These results are, in general, similar to those shown by the orchard test with growing apples still attached to the tree when the branch bearing them was kept under water for 4 days (table 1). The water treatment of detached apples resulted in much cracking, and most of the badly cracked fruit showed relatively large gains in weight.

As in the orchard experiment on cracking of attached apples under water, it is clear that this 2-day water treatment of mature, detached fruits did produce cracking, that it was more apt to do so when the rate of enlargement was rapid than when it was slow, and that severity of the cracking produced generally was greater for those fruits that enlarged more rapidly. Since the cut end of the stem and the opening of the calyx region were sealed, it is evident that weight gain must have been due to water absorption through the skin. This observation furnished additional evidence that such direct absorption is to be expected whenever the apple skin is kept externally wet for a sufficient time. The apples that failed to crack also absorbed water, and one of these (no. 18) showed the most rapid absorption of all.

EXPERIMENTS ON VASCULAR PRESSURE AND CONDUCTION

A few experiments were performed with reference to possible relations between the occurrence of cracking and conditions that might influence the supply of water and other substances to the fruit. These are briefly described below.

VASCULAR SAP PRESSURE IN ATTACHED BRANCHES

Mercury manometers were connected to small glass cylinders filled with water and fastened over the cut surfaces where tip portions of attached branches had been removed. These manometers invariably

showed maintained pressure deficits in the attached branches. It was thus apparent that the hydrostatic pressure of the vascular sap generally was considerably less than the gas pressure of the surrounding air. This was true even when cracking of the fruits was in progress, but the pressure deficit was smaller in periods of low evaporation rates than at drier times. Degrees of suction amounting to from 143 mm of a mercury column to 400 mm were observed. The method used is of course inadequate to show maximal pressure deficits, and it seems safe to suppose that the deficits recorded were generally smaller than the actual current deficits in uninjured trees or even in the cut branches some distance back of the cut surfaces. We may, in any event, conclude that vascular hydrostatic pressure in a branch when apples were cracking generally was considerably less than the current barometer reading.

These manometer experiments indicate clearly that there was no general vascular pressure excess in these trees, which might, as some writers seem to think, act to force water into the fruits at times of cracking. There was no evidence of positive excess pressure derived from the roots and transmitted throughout the tree, such as seems to have been postulated whenever the term "root pressure" has been employed in the elaboration of hypotheses concerning cracking of fruits and other phenomena connected with plant-water relations.

EXCESSIVE WATER PRESSURE ARTIFICIALLY PRODUCED IN BRANCHES

Some unsuccessful laboratory attempts were made to cause apples to crack by forcing water under excessive pressure into the cut basal ends of branches bearing fruit. The cut end of the branch, where the latter had been detached from the tree, was tightly sealed into the large opening of a tubulated filter flask containing water enough to cover the cut surface to a height of about 5 cm, and a Schrader valve, such as is used in automobile tires, was securely fastened to the tubulature by means of a bit of rubber tubing and a wire ligature. Then a hand-operated compression air pump was connected to the valve and air was pumped into the flask to increase the gas pressure in its upper portion and so force water into the branch. Pressures of from 5 to 10 pounds were employed. No cracking of the fruit resulted from this treatment, in which branch and fruit were exposed to the air of the laboratory. This experiment was not tried with branch and fruit surrounded by water-saturated air.

Within a few minutes after pressure had been applied to the system, water dripped rapidly from surfaces where ends of lateral twigs had been cut off, and more slowly from cut midribs of leaves; but no exudation appeared at surfaces where fruit stems had been cut. These tests lasted from 30 minutes to 3 hours. The results would be more significant if the artificially produced excessive vascular pressure had been applied for a longer time and if transpiration had been prevented, as by keeping leaf and fruit surfaces continuously wet.

DYE SOLUTIONS ABSORBED THROUGH CUT ENDS OF BRANCHES STILL ATTACHED TO TREE

Aqueous dye solutions were fed into the cut ends of small branches from 1 to 2 cm in diameter, and dye penetration into nearby fruits was observed. Both acid fuchsin and methyl blue were used with

equally satisfactory results. The solution entered under the influence of vascular-pressure deficit, no excess pressure being applied. The color contrast of either dye with the whitish flesh of the apple was quite striking, the dyed regions standing out clearly when the fruit was cut open. The dyes appeared in the regions of the vascular bundles and to a greater or lesser extent in contiguous meristematic tissues. Methyl blue was detected in very small veinlets close under the skin.

A striking feature of the penetration and accumulation of these dyes was their consistently greater concentration in regions subjacent to abnormal peripheral tissues, such as russeted areas, apple-scab lesions, and old cracks. Although sap movement through the vessels was probably somewhat more rapid and perhaps somewhat more extensive than was indicated by dye accumulation in the cell walls, yet there is no reason to suspect that the dye ever moved more rapidly than the sap that carried it, and the final presence of more dye in one region than in another surely means that sap had moved into the more intensely stained parts more rapidly than to other parts that showed less dye accumulation. It therefore appears that sap flow to the modified or injured peripheral regions just mentioned was much more rapid than to uninjured peripheral tissues, and this apparently indicates, in turn, that transpiration was more intense from the injured tissues than from other peripheral regions of the fruit. Baker (1) found that transpirational water loss through the corky tissue of russeted regions of apples was considerably greater than through normal epidermis and cuticle. Since the tissues just beneath the corky regions do not wither as long as the apples are on the tree, but remain turgid and healthy, this observation implies a more rapid movement of water into those regions.

From what has just been said it may be concluded that, except when transpiration is almost stopped, russeted, cracked, or otherwise locally injured peripheral tissues of the apple fruit transmit water from vessels to periphery more rapidly than do uninjured tissues, whose resistance to transpirational water loss is apparently greater; i. e., for the cells of the injured tissue both entrance of water and water loss generally are more rapid than for the cells of corresponding uninjured tissue. Many students have presented evidence in support of the general observation that larger bundles and more vessels develop in regions adjacent to rapidly transpiring surfaces than in other regions; in some unknown manner it appears that exceptionally rapid flow of sap through a developing bundle causes the formation of more vessels, or of more efficient ones, than would have been formed had the rate of sap flow been much slower. On the basis of this proposition it may be supposed that vascular resistance to sap movement is apt to be exceptionally low in those bundles that lead to the injured regions of the apple. If such is the case, vascular sap may be supposed to reach those regions with somewhat less hydrostatic pressure deficit than prevails in other regions, especially in times of very slow or negligible foliar transpiration, when vascular-pressure deficit must be exceptionally low throughout the whole tree. In periods of rapid transpiration the suction (or even liquid traction) arising therefrom should act to retard cell absorption everywhere. These considerations of logical possibilities may be assembled to furnish a hypothesis that

may explain, at least in part, why injured portions of apple periphery proved to be more susceptible to cracking than other portions, and why outbreaks of cracking and the occurrence of the most severe cracking were confined to periods when the general transpiration rate was unusually low.

INTERNAL CONDITIONS OF FRUIT IN RELATION TO CRACKING

GENERAL RELATIONS BETWEEN EXTERNAL AND INTERNAL INFLUENCES

As has been pointed out, cracking of the fruit is of much more common occurrence in some apple varieties than in others. When cracking is prevalent in an orchard some trees usually are affected much more than others and some branches often are affected more than others on the same tree. Even on those fruiting branches exhibiting the most severe cracking when external conditions tend to promote this injury, some apples usually fail to crack. In the present study some apples were always found to be uninjured at harvest time, even on trees that exhibited high percentages of cracking.

It is therefore evident that cracking of individual fruits must be determined not alone by the occurrence of favorable weather conditions but also by some internal conditions effective within the fruit at times when the weather is favorable to cracking. The phenomenon of cracking is to be considered as the result of some combination or concatenation of circumstances, some of which may be traced almost directly to environmental fluctuations, while others appear to result from antecedent physiological processes of nutrition, growth, and development within the apple tree or within the fruit itself. In apples the formation of a crack shows that the fruit tissues involved, and perhaps other tissues also, were, when cracking occurred, unusually susceptible to the current cracking influence of the environmental complex, and it shows just as clearly that the conditions of the environment were such as to permit or facilitate crack formation in tissues exhibiting this particular kind and degree of susceptibility. Consequently the problem of the causation of cracking cannot be considered satisfactorily in terms of environmental conditions alone or in terms of internal conditions alone; both external and internal influences need to be taken into account.

The preceding sections of this paper deal mainly with cracking as it appeared to be related to environmental conditions, although attention is given to some internal conditions: e. g., vascular sap pressure and injured or modified regions of the fruit periphery. The present section deals with some observations on additional internal conditions: viz, abnormalities of peripheral tissues and the freezing-point depression of cortical tissues.

ABNORMALITIES OF PERIPHERAL TISSUES

As has been noted, cracking is less likely to occur on sound apples than on those with some abnormality of the peripheral tissues. Cracks are found much more often on portions of the fruit periphery that are marked by russetting, by scab lesions, or by sunburn than on other portions of the fruit. This point is illustrated by figure 1. Both in 1932 and 1933 the lesions that appeared earliest in any outbreak of cracking were predominantly on these modified parts of the

fruit surfaces. In 1933, 88 percent of the cracks formed on the fruits of one tree were directly associated with russeted skin, sunburn, or scab spots. The remaining 12 percent were in fruit surfaces that appeared otherwise to be perfectly healthy and unmodified. Cracks on otherwise sound fruits most often were on the cheek that was most exposed to sunlight. It should be emphasized, however, that cracking in apparently normal, uninjured peripheral regions has shown the same relationships to environmental fluctuations as was shown by cracking in the regions of modified periphery. Cracking in abnormal regions of the fruits as observed in these experiments is not to be regarded as a special form of the injury dependent on the abnormalities themselves, that is, the abnormalities merely render affected portions of fruits more susceptible to the injury than normal portions when environmental influences tend to promote cracking in both.

FREEZING-POINT DEPRESSION OF FRUIT TISSUES

The observation that cracking was much more apt to occur on the most exposed side of an apple, especially when the skin and adjacent tissues on that side were modified by high coloration, sunburn, or russetting, leads to the suggestion that the turgor pressure in the tissues underlying the modified or injured portion of the periphery might be greater than that of corresponding tissues underlying unmodified peripheral regions of the same fruit. Brooks and Fisher (3) found that on very hot summer days the sunny side of an apple had a temperature from 10° to 16° F. higher than the shaded side. Juice expressed from the most exposed quarter of the fruit generally showed greater freezing-point depression than juice similarly expressed from the opposite quarter, and this difference was greater as the exposure of the first quarter was more pronounced. This was taken to indicate higher osmotic concentration in the cell sap of the tissues on the exposed side, which might be due, for example, to the higher temperature or to the more intense insolation on that side during periods of sunshine.

In a later paper (4) the same authors reported that exceptionally high osmotic values were indicated for tissue from the sunburned side, when one side was so injured; also that there seemed to be positive correlation between evaporation from a Livingston standardized white-sphere atmometer and the osmotic value of juice from apples in different parts of the tree, both being greatest in the position of greatest exposure to sunshine. A similar effect of seasonal conditions on the crop as a whole is suggested by Caldwell's (6) analyses of apple juice, which show, for many varieties, on unusually high sugar content in seasons with temperature and sunshine considerably above normal.

By means of the tissue-cup freezing-point method (28) many comparisons were made between sample cups from different regions of the same apple, especially between tissue samples from regions underlying unmodified skin areas and other tissue samples from regions underlying areas of high coloration, sunburn, or russetting. In some instances the more susceptible region of the apple tested had already cracked; that is, tissue underlying one or more newly developed cracks was compared, with reference to freezing-point depression, with tissue underlying an intact area of the same apple. After making the usual correction necessitated by supercooling, each freezing-point

depression was translated into terms of osmotic concentration in atmospheres by reference to the published table of Harris and Gortner (15). It should be emphasized that this index refers to the sample cup of tissue as a whole, not to its cell sap alone. It represents the capacity of the tested tissue to retain water against the influence of drying agencies, and it embraces not only the resilient pressure of stretched cell walls (which tends to squeeze water out of the cells), but also the osmotic value of the cell sap and of the liquid held in the walls themselves, as well as the imbibitional values of cell colloids and cell walls (which together act to retard water loss).

Results derived from 52 different apples of the Stayman Winesap variety, picked from eight different trees, are presented in table 5. Two values are shown for each apple, derived, respectively, from two tissue-cup samples taken from opposite sides of the fruit axis, with the longitudinal dimension of each cup parallel to the latter. The tissue of each cup was thus derived from a region opposite the middle portion of the apple core and about half-way between the core and the cheek periphery on that side. One of each pair of cup samples was taken from beneath a cheek area that showed modification of some sort, while the other sample was taken from beneath unmodified cheek periphery. Five kinds of visible modification or injury of peripheral tissue were recognized: (1) highly colored skin, uncracked; (2) sunburned skin, uncracked; (3) russeted periphery, recently cracked; (4) russeted periphery, uncracked; and (5) recently cracked periphery otherwise apparently unmodified.

It is seen at once that the tissue samples derived from beneath modified or cracked areas of the fruit periphery showed significantly higher osmotic values, in almost every test, than were shown by the samples derived from beneath unmodified areas. Since highly colored, sunburned, or russeted areas of the fruit surface have been found to be exceptionally prone to crack, this remarkable osmotic relation lends definite support to the supposition that cracking may result from excessive enlargement or excessive tissue pressure in localized subperipheral regions of the fruit cortex; for, other conditions being the same, regions of exceptionally high osmotic value should absorb water, and therefore enlarge, more rapidly than neighboring regions of lower osmotic value. The data of table 5 therefore indicate a reason why cracking is most likely to occur over regions of the cortex that are superficially marked by high coloration, sunburn, or russetting. The averages for the several sections of table 5 lead to the same conclusions as are reached from more detailed study. For every group of comparisons the average for the unmodified is smaller than for the modified areas.

[illegible]

Pronounced localization of high tissue pressure is clearly indicated, and it seems likely that differences within the same apple might be found to be even greater than those shown in table 5 if it were possible to employ smaller tissue samples in freezing-point determinations of this kind. An attempt was made to chart the pressure variations somewhat more in detail through the use of 5 tissue cups from each apple instead of 2, the positions for sampling being symmetrically arranged around the fruit axis. The results of these more elaborate tests were in excellent agreement with those set forth in table 5. A representative set of data from five samples is shown in figure 5. The small circles represent the positions of the tissue cups studied, and the numerical values within these circles are osmotic values like those given in table 5. Apparently there was a tissue-pressure gradient in the subperipheral cortex of the fruit, between a high pressure underneath the more exposed peripheral area and a much lower pressure in the corresponding region on the opposite side of the fruit axis.

When the several local osmotic pressures found by employing 4 or 5 tissue samples from the same apple were averaged, to give an approximation of the mean-tissue pressure of the whole fruit, it was found that this mean value showed no relation to cracking. Some apples with low means were badly cracked while some with much higher means showed no cracking. It is therefore indicated that the internal conditions that favor or hinder cracking are not to be studied by means of freezing-point values derived from whole apples or from pulp or juice obtained by grinding or pressing the entire fruit.

There was apparently no relation between a tendency to crack and the absolute tissue pressure of the subperipheral cortex; cracking might be associated with a local tissue pressure of only 15 atmospheres in one apple, while a local pressure of 20 atmospheres or more might be found in another apple that was still sound. It seems that cracking must be related, in general, not to the local subperipheral pressure itself but to the magnitude and distribution of the pressure gradient just mentioned. In short, one of the main internal conditions leading to crack formation appears to be the occurrence of an excessive tissue pressure in a restricted area of the fruit cortex, accompanied by much lower pressures in neighboring regions.

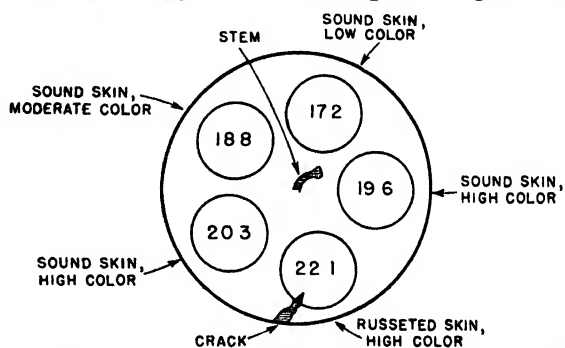


FIGURE 5.—Graphic representation of osmotic values, in atmospheres, of tissue samples from different portions of one Stayman Winesap apple.

SUMMARY AND CONCLUSIONS

This paper deals with cracking of the peripheral tissues of Stayman Winesap apples, which is considered in relation to current environmental and internal conditions. The discussions and views presented

are based on (1) observational studies carried out on cracking under natural conditions in an experimental orchard at Kearneysville, W. Va., (2) orchard experiments in which some environmental conditions were artificially modified, and (3) special laboratory studies.

[Sudden, great increase in soil-moisture content has been suggested by some writers as a probable major factor contributing to cracking of fruits in general.] but the observations and experiments recorded in the present paper fail to furnish any evidence in support of this hypothesis. [Pronounced fluctuations in soil-moisture content, greater and more rapid than are ordinarily met with in orchards, showed no relationship to either the occurrence or the severity of cracking.]

[Under natural orchard conditions the occurrence of cracking was always found to be associated with very low rates of evaporation. Rainfall was naturally confined to periods so characterized, but cracking was clearly correlated with the slow evaporation rather than with the rainfall. Thus, extensive cracking occurred in several instances of prolonged slow evaporation rates when there had been no rainfall for from 36 hours to 6 days; and in other instances no cracking was observed after heavy rains when the accompanying low evaporation rates were of short duration.] An outbreak of cracking was generally preceded by a period of 6 hours or more during which a standardized black porous porcelain atmometer sphere with automatic recorder showed no measurable water loss and the water-saturation deficit of the air was very low. This correlation may be taken to indicate that outbreaks of cracking were generally brought about by greatly depressed transpiration, maintained for 6 hours or more. [During a period of very slow transpiration the water supply available to the fruits should be very abundant, permitting unusually rapid enlargement of fruit cortex if other conditions are favorable for tissue swelling. If, at the same time, rain water is being absorbed appreciably through the peripheries of fruits and leaves, swelling of the fruit cortex should be still further increased.]

Mercury manometers connected to attached branches where the branch tips had been cut off showed vascular suction at all times, even while nearby fruits were in process of cracking. No evidence was found to favor the supposition that excessive vascular pressure (commonly referred to as "root pressure") was generally present throughout these apple trees at any time; atmospheric pressure apparently always exceeded the hydrostatic pressure within the vessels of the branches. [Attempts to produce cracking artificially by forcing water, under 5 to 10 pounds of excessive pressure per square inch, into the cut ends of detached branches bearing fruit failed when branch and fruit were exposed to the air of the laboratory and the treatment was continued for 3 hours or less.]

[Since change in soil-moisture content did not noticeably influence cracking in this study it appears that precipitation was not influential through wetting of the soil. Rainfall may be influential in other ways, however, for when fruit and leaf surfaces are more or less thoroughly covered with water during periods of rain, considerable water absorption may occur through those surfaces, while transpiration is correspondingly retarded or prevented. When rain was artificially diverted from large branches some of the apples on those branches cracked, although they and the accompanying leaves were thus kept superficially dry, which seems to indicate that the presence

of a film of water on foliage or fruit, or both, was not a necessary condition to promote cracking. On the other hand, when a fruit-bearing branch, still attached to the tree, was kept under water for several days the submerged apples showed severe cracking; and cracking was also artificially induced when detached apples, with stems and calyxes sealed, were kept under water for several days, suggesting that long-continued wetting of fruit and foliar surfaces by rain might aggravate the tendency to crack when other conditions tend to promote this injury. There is no doubt that the apples of this study were able to absorb water at a considerable rate when their skins were kept superficially wet. Enclosing attached apples in paper bags to prevent wetting of the skins by rain greatly reduced cracking in the apples so treated after the bags had been in place for 3 or 4 weeks; in that time, however, the internal conditions of the bagged apples may have changed greatly.

The occurrence and severity of cracking were not shown to be related in any way to air-temperature fluctuation.]

Not all the apples on a branch or tree cracked when the weather conditions were favorable to cracking, and it is evident that some fruits were, at any time, more susceptible to cracking than others. A period of little or no evaporation apparently induced cracking of the currently most susceptible fruits only. With the recurrence of low evaporation rates additional fruits cracked, suggesting that this susceptibility of individual apples was likely to increase throughout the season. Some fruits cracked early in the season while others still remained sound at harvest time. A study of certain internal conditions that may have influenced predisposition to crack led to several apparently significant observations, as follows:

In a great majority of observed cases of cracking the injury was initiated in a region characterized by some form of visible abnormality or modification of the peripheral tissues of the fruit: viz, russetting, scab lesions, sunscald, or unusually high coloration. Such modifications usually were found on the side of the fruit that had experienced the greatest exposure to sun, wind, and spray injury. In russeted areas many minute cracks appeared to have been progressively formed and then closed by cork tissues, leaving the fruit periphery more or less roughened and mechanically weak in such regions.

Apparently the immediate mechanical cause of cracking is a form of tissue strain by which the skin and the adjacent tissues in a restricted area of the apple are excessively stretched because of enlargement in deeper lying tissues beneath the same area. With weather conditions suitable for crack formation, it seems that rupture occurs in the most susceptible peripheral areas, where the sub-peripheral pressure exceeds the limit of extensibility of the outer tissues. Thus the formation of a crack is determined partly by the mechanical strength of the peripheral tissue and partly by the magnitude of the pressure exerted by the enlarging tissue beneath.

It was observed that, among trees and branches otherwise apparently comparable, cracking was more pronounced and extensive when the foliage was sparse than when it was dense. This difference may have been related to the greater incidence of sunscald, russetting, and intense coloration in the fruits of trees and branches with poor foliage.

When attached apples were kept under water, cracking generally occurred first and most severely in those individuals that showed the most rapid rates of enlargement, which suggests that rapid enlargement of the entire fruit may tend to produce cracking when other conditions are suitable. But some slowly enlarging fruits cracked while some rapidly enlarging ones remained sound, and it seems that the excessive tissue swelling that resulted in a crack was sometimes effective only beneath the immediate peripheral region where rupture was about to occur.

Freezing-point measurements of osmotic values within the apple tissues showed, almost without exception, that tissue samples from regions beneath modified areas of the fruit periphery had significantly higher pressures than had similar samples from unmodified areas. For example, an apple with a russeted area on one side showed a high osmotic value beneath that area and a much lower osmotic value beneath the corresponding area on the opposite side of the fruit, with a gradient between these two regions. It therefore appears that the russeted or otherwise modified region was characterized not only by mechanical weakness of the periphery but also by exceptionally high tissue pressure in the deeper lying cortex. Both of these characteristics would be expected to favor cracking.

When aqueous dye solutions were fed into the cut ends of attached branches bearing fruit, the solutions were absorbed into the branches and carried into nearby apples, where the dyes became most concentrated in those parts of the peripheral regions characterized by russetting, scab lesions, or previously formed and more or less suberized cracks. Movement of vascular sap into the tissue beneath these modified regions and the passage of water out of them by transpiration were more rapid than elsewhere. With a low evaporation rate and consequent slow transpiration, it appears that the cortex beneath modified periphery may receive water more freely than other parts of the same fruit. This may promote excessive swelling beneath the modified areas.

LITERATURE CITED

- (1) BAKER, C. E.
1931. A STUDY OF SKIN STRUCTURE OF THE GRIMES APPLE AS AFFECTED BY DIFFERENT TYPES OF INJURY. *Amer. Soc. Hort. Sci. Proc.* (1930) 27: 75-81, illus.
- (2) BOUSSINGAULT, J.
1873. SUR LA RUPTURE DE LA PELLICULE DES FRUITS EXPOSÉS À UNE PLUIE CONTINUE. ENDOSMOSE DES FEUILLES ET DES RACINES. *Ann. Chim. et Phys.* (4) 29: 360-367. [Abstract in *Just's Bot. Jahresber.* 1: 253. 1873.]
- (3) BROOKS, C., and FISHER, D. F.
1926. SOME HIGH-TEMPERATURE EFFECTS IN APPLES: CONTRASTS IN THE TWO SIDES OF AN APPLE. *Jour. Agr. Research* 32: 1-16, illus.
- (4) ——— and FISHER, D. F.
1926. WATER-CORE OF APPLES. *Jour. Agr. Research* 32: 223-260, illus.
- (5) BURKE, R. T. A., and MCCALL, H. F.
1919. SOIL SURVEY OF WASHINGTON COUNTY, MARYLAND. U. S. Dept. Agr., Bur. Soils Adv. Sheets Field Oper. 1917, 46 pp., illus.
- (6) CALDWELL, J. S.
1928. CHEMICAL COMPOSITION OF APPLE JUICES AS AFFECTED BY CLIMATIC CONDITIONS. *Jour. Agr. Research* 36: 289-365, illus.
- (7) CAMPBELL, J.
1928. CRACKING OF DUNN'S AND COX'S ORANGE APPLES. *New Zeal. Jour. Agr.* 37: 85-86.

- (8) CHANDLER, W. H.
1925. FRUIT GROWING. 770 pp., illus. Boston, New York [etc.]
- (9) COIT, J. E.
1917. CITRUS FRUITS, AN ACCOUNT OF THE CITRUS FRUIT INDUSTRY, WITH SPECIAL REFERENCE TO CALIFORNIA REQUIREMENTS AND PRACTICES AND SIMILAR CONDITIONS. 520 pp., illus. New York.
- (10) DEVAUX, H.
1900. RECHERCHES SUR LES LENTICELLES; ÉTUDE SUR LES CONDITIONS PHYSIOLOGIQUES DE L'ACCROISSEMENT ET DE LA DIFFÉRENCIATION DE LA CELLULE ET DES TISSUS. *Ann. Sci. Nat., Bot.* (8) 12: 1-240, illus.
- (11) FAWCETT, H. S., and LEE, H. A.
1926. CITRUS DISEASES AND THEIR CONTROL . . . 582 pp., illus. New York.
- (12) GARDNER, V. R., BRADFORD, F. C., and HOOKER, H. D.
1922. THE FUNDAMENTALS OF FRUIT PRODUCTION. 686 pp., illus. New York.
- (13) GOODWIN, B. C.
1929. BLISTER DISEASE OR CRACKING OF APPLES: SUCCESSFUL REMEDIAL MEASURES IN NELSON DISTRICT. *New Zeal. Jour. Agr.* 39: 305-307.
- (14) GRAEBNER, P.
1920. LEHRBUCH DER NICHTPARASITÄREN PFLANZENKRANKHEITEN. 333 pp., illus. Berlin.
- (15) HARRIS, J. A., and GORTNER, R. A.
1914. NOTES ON THE CALCULATION OF THE OSMOTIC PRESSURE OF EXPRESSED VEGETABLE SAPS FROM THE DEPRESSION OF THE FREEZING POINT, WITH A TABLE FOR THE VALUES OF P FOR $\Delta = 0.001^{\circ}$ TO $\Delta = 2.999^{\circ}$. *Amer. Jour. Bot.* 1: 75-78.
- (16) HARTMAN, H., and BULLIS, D. E.
1929. INVESTIGATIONS RELATING TO THE HANDLING OF SWEET CHERRIES WITH SPECIAL REFERENCE TO CHEMICAL AND PHYSIOLOGICAL ACTIVITIES DURING RIPENING. *Oreg. Agr. Expt. Sta. Bull.* 247, 38 pp., illus.
- (17) HEALD, F. D.
1933. MANUAL OF PLANT DISEASES. Ed. 2, 953 pp., illus. New York and London.
- (18) LIVINGSTON, B. E.
1911. A RADIO-ATMOMETER FOR COMPARING LIGHT INTENSITIES. *Plant World* 14: 96-99.
- (19) ———
1915. ATMOMETRY AND THE POROUS CUP ATMOMETER. *Plant World* 18: 21-30, 51-74, 95-111, 143-149, illus.
- (20) RIXFORD, G. P.
1918. SMYRNA FIG CULTURE. *U. S. Dept. Agr. Bull.* 732, 43 pp., illus.
- (21) SAWADA, E.
1931. STUDIES ON THE CRACKING OF CHERRIES. *Agr. and Hort.* 6: 865-892. [In Japanese, with English summary.]
- (22) SCHILBERSZKY, K.
1918. HIPERTROFOS PARASZEMÖLESÖK ALMAGYÜMÖLÖSKÖN. (HYPER-TROPHE LENTIZELLEN AUF APFELFRÜCHTEN). *Bot. Közlemenyek* 17: 93. [Reviewed by Matouschek in *Ztschr. Pflanzenkrank.* 29: 249. 1919.]
- (23) SORAUER, P.
1922. MANUAL OF PLANT DISEASES. Transl. by F. Dorrance. Ed. 3, v. 1, illus. [Wilkes-Barré, Pa.]
- (24) SWINGLE, C. F.
1929. A PHYSIOLOGICAL STUDY OF ROOTING AND CALLUSING IN APPLE AND WILLOW. *Jour. Agr. Research* 39: 81-128, illus.
- (25) TRACY, W. W.
1907. TOMATO CULTURE; A PRACTICAL TREATISE ON THE TOMATO, ITS HISTORY, CHARACTERISTICS, PLANTING, FERTILIZATION, CULTIVATION IN FIELD, GARDEN, AND GREENHOUSE, HARVESTING, PACKING, STORING, MARKETING, INSECT ENEMIES AND DISEASES, WITH METHODS OF CONTROL AND REMEDIES, ETC., ETC. 150 pp., illus. New York.

-
- (26) VEIHMEYER, F. J.
1927. SOME FACTORS AFFECTING THE IRRIGATION REQUIREMENTS OF
DECIDUOUS ORCHARDS. *Hilgardia* 2: [125]-290, illus.
- (27) ———
1929. AN IMPROVED SOIL-SAMPLING TUBE. *Soil Sci.* 27: 147-152.
- (28) VERNER, L.
1934. A SIMPLIFIED METHOD OF DETERMINING FREEZING-POINT DEPRES-
SIONS OF APPLE TISSUE WITH THE BECKMANN APPARATUS.
Amer. Soc. Hort. Sci. Proc. 31: 33-34.
- (29) ——— and BLODGETT, E. C.
1931. PHYSIOLOGICAL STUDIES OF THE CRACKING OF SWEET CHERRIES.
Idaho Agr. Expt. Sta. Bull. 184, 15 pp., illus.

POTATO BEETLE SEPTICEMIA¹

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INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), widely known as a pest of potato, also feeds on tomato, tobacco, eggplant, pepper, belladonna, henbane, and other solanaceous plants. It is, therefore, a pest of major importance. In an earlier note the writer² reported the existence of a bacterial disease of this insect. The present paper describes the disease and incorporates observations made on it from 1921 to 1929.

NAME OF THE DISEASE AND THE PATHOGENIC BACILLUS ASSOCIATED WITH IT

The potato beetle disease and the disease produced experimentally in insects inoculated by puncture with material from larvae sick or dead of the disorder are characterized by a marked septicemia. This suggested the name "potato beetle septicemia." The names "hornworm septicemia"³ and "cutworm septicemia"⁴ were coined earlier for similar diseases of hornworms and cutworms, respectively. A similar disease of grasshoppers, first described by D'Herelle⁵, has received much attention.

The pathogen associated with the grasshopper disease is *Bacillus (coccobacillus) acridiorum* D'Herelle, the one associated with the hornworm disease is *B. sphingidis* White, and the one associated with the cutworm disorder is *B. noctuarum* White. In line with this method of choosing specific names for these organisms, the name *B. leptinotarsae* was given by the writer⁶ to the pathogenic species associated with potato beetle septicemia. All these species are closely related and are members of a large group of organisms affecting insects which may be termed the "septicemia" group.

SYMPTOMS

Larvae affected by potato beetle septicemia are at first sluggish but soon become motionless. Their appetite is impaired, and they soon cease to feed. Usually they fall to the ground when moribund or dead, although dead ones are occasionally found adhering to the food plant (fig. 1, A). The appearance of sick larvae and of those recently dead is very similar to that of healthy ones. Soon after death the reddish tint of healthy larvae changes to a brownish gray. The

¹ Received for publication Apr. 23, 1935, issued September, 1935.

² WHITE, G. F. POTATO BEETLE SEPTICEMIA, WITH THE PROPOSAL OF A NEW SPECIES OF BACTERIUM. Ent. Soc. Wash. Proc. 30: 71-72. 1928.

³ ——— HORNWORM SEPTICEMIA. Jour. Agr. Research 26: 477-486, illus. 1923.

⁴ ——— CUTWORM SEPTICEMIA. Jour. Agr. Research 20: 487-496, illus. 1923.

⁵ HERELLE, F. D' SUR LA PROPAGATION, DANS LA RÉPUBLIQUE ARGENTINE, DE L'ÉPIZOOTIE DES SAUTERELLES DU MEXIQUE. Compt. Rend Acad. Sci. [Paris] 164 623-625. 1912.

⁶ WHITE, G. F. See footnote 2.

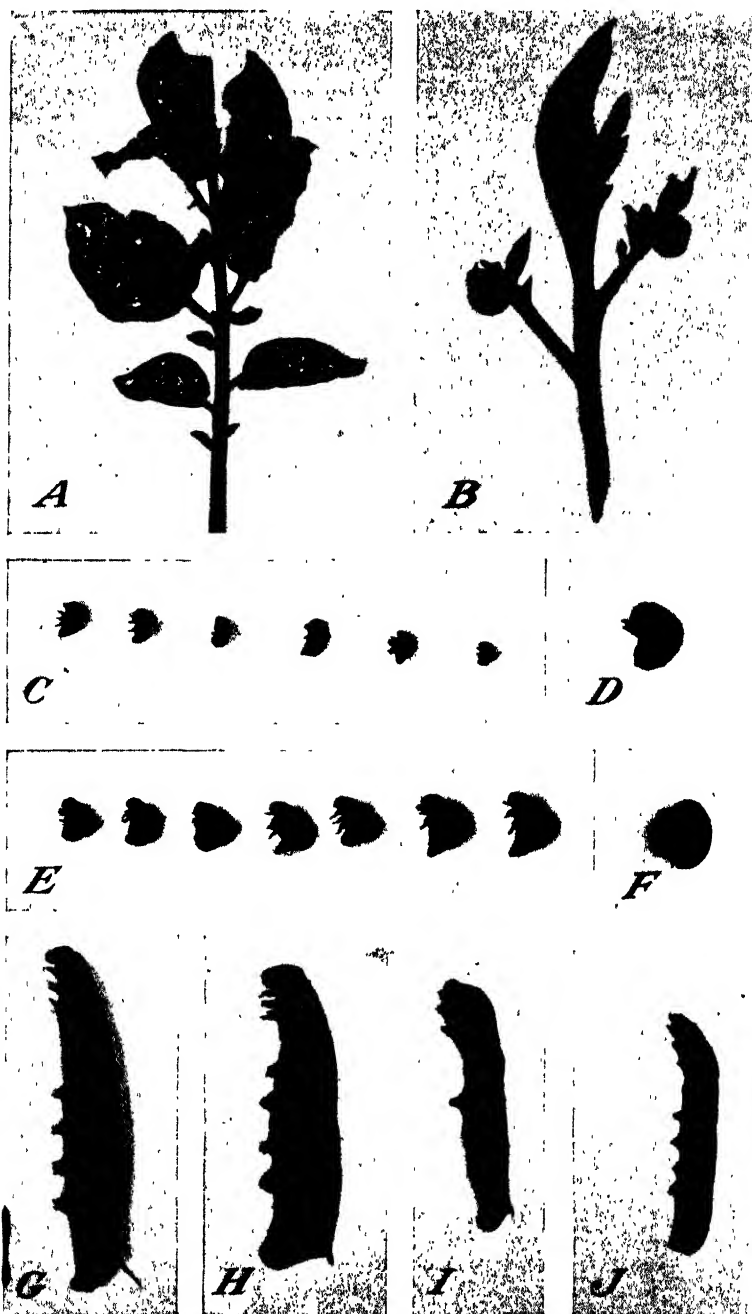


FIGURE 1.—Potato beetle septicemia: A, Larva sick of potato beetle septicemia, natural infection; B, larvae sick or dead of potato beetle septicemia, inoculated experimentally; C and D, larvae dead of potato beetle septicemia, natural infection. E and F, larvae and adult, respectively, dead of potato beetle septicemia, inoculated experimentally; G, catalpa sphinx larva inoculated with *Bacillus leptinotarsae*; H, hornworm inoculated with *B. leptinotarsae*; I, silkworm inoculated with *B. leptinotarsae*; J, cutworm inoculated with *B. leptinotarsae*.

black markings remain unchanged. As decay advances the color deepens, becoming a dark gray, dark brown, or nearly black.

The dead larvae retain for a while the surface contour of healthy ones; but as drying takes place, the surface of the body becomes roughened, and within a week the remains are a shriveled, dark, brittle mass (fig. 1, *C, D*). During most of the period of decay the resistance of the body wall to tearing is similar to that of healthy larvae. The decaying mass is nonviscid. Usually the odor is slight and not particularly disagreeable.

TECHNIC

The laboratory methods used in these studies were in general similar to those commonly employed in disease work. Good results were obtained from the incubation of cultures at room temperature or at approximately 30° C. In addition to the usual laboratory animals, larvae and adults of the potato beetle, silkworms (*Bombyx mori* L.), hornworms (*Phlegethontius quinquemaculata* Haw. and *P. sexta* Joh.), various species of cutworms and white grubs, and caterpillars of the catalpa sphinx (*Ceratomia catalpae* Bdv.) were used for experimental purposes. The puncture and feeding methods employed in earlier studies⁷ were followed. When a fine needle was used and care was exercised to limit the injury caused to the body wall, death resulted only occasionally from trauma inflicted by puncture inoculations.

Potato plants growing in pots served well as food for the larvae and adult potato beetles after they had been inoculated. Following puncture inoculations the insects were placed immediately on the potted plants. In the feeding inoculations the plants were sprayed with an aqueous suspension of disease material or with cultures, and the insects to be inoculated were placed on them while the plants were still wet. In inoculations by immersion the insects were immersed in a beaker containing the culture and afterward placed on a growing potato plant.

A cardboard or other suitable paper was fitted about the base of the plant for collecting dead or sick insects as they fell from the plant or otherwise left it. The mature, unaffected larvae leaving the plant may escape and, reaching the soil within the pot, burrow into it for pupation. Moribund larvae and even dead ones occasionally are found adhering to the plant (fig. 1, *B*), as was observed in potato beetle septicemia encountered in nature.

POST-MORTEM CHANGES IN EXPERIMENTAL INSECTS

The changes occurring in the bodies of insects that die from experimental infection with cultures of *Bacillus leptinotarsae* vary somewhat with the different insect species used, and slightly with the different cultures employed in the inoculations. Temperature and moisture tend to modify the speed of the changes that occur and to some extent their character. Potato beetle larvae (fig. 1, *E*) that died from experimental inoculation showed post-mortem changes similar to those observed in larvae that died of the disease in nature.

⁷ WHITE, G. F. See footnote 3.

Silkworms (fig. 1, *I*) in the fifth instar inoculated with disease material of infected insects or with pure cultures of *Bacillus leptinotarsae* die within 24 hours when the temperature of the room is 30° C. or higher. Soon after death the remains turn a light brown, which is preceded at times by a slightly greenish hue. At first the color changes are not uniform over the entire body but by the end of 24 hours they become so. The hue is then brown which gradually darkens until it becomes almost black. The body wall soon yields easily to tearing, and the contents become a moist, thick, brown mass. When allowed to dry the remains become brittle by the end of a week. The odor from the bodies is not particularly offensive when they are left in the open, but becomes so when they are confined.

The post-mortem changes in hornworm larvae (fig. 1, *H*) that have died from experimental infection with *Bacillus leptinotarsae* are similar to those noted for silkworms.

The bodies of catalpa sphinx larvae (fig. 1, *G*) that have died from experimental inoculation with the bacillus soon turn brown. During the first day of post-mortem changes the body wall will usually support the weight of the dead larva, but later it becomes less resistant.

The bodies of cutworms (fig. 1, *J*) dead from experimental infection become a deep brown within 2 days. The body wall is then easily torn, and the decaying tissues are a moist, thick, brown mass which is brittle after it becomes dry.

White grubs that have died from puncture inoculations may first present a mottled appearance, but later they become a brown which may appear almost black.

OCCURRENCE OF POTATO BEETLE SEPTICEMIA

A disease of the septicemia group may be suspected when cultures made from insects dying in nature yield a luxuriant growth of numerous colonies of a small actively motile bacillus. Such a disease is further indicated when silkworms or other experimental insects inoculated by puncture with the bacillus die within 1, 2, or 3 days and death is accompanied by a septicemia and followed by post-mortem changes characterized by a dark-brown color and softening of the remains.

On June 10, 1921, Doris H. Blake, of the Bureau of Entomology, while engaged in biological studies on the potato beetle, found larvae in Washington, D. C., that were dead from a cause unknown to her. Two silkworms inoculated by puncture with an aqueous suspension of one of these larvae died within 24 hours. Five silkworms inoculated with the blood from the two dead silkworms died also. The blood of these recently dead worms contained many short motile rods. Agar plates made from the bodies yielded numerous gray colonies of an actively motile bacillus. The bodies became soft and turned brown. The presence of a disease of the septicemia group was therefore indicated.

On August 29, 1921, W. H. White, also of the Bureau of Entomology, collected sick potato beetle larvae from potato plants at Arlington, Va. A short, actively motile bacillus that produced a gray growth was isolated from each of the larvae that were cultured. Two healthy potato beetle larvae were inoculated with cultures from

each sick larva. All the inoculated larvae died within 2 days. The indications, therefore, were that this disease also belonged to the septicemia group.

On August 15, 1922, the writer collected about 50 potato beetle larvae, some sick and others recently dead, from potato plants grown on the plot in Washington where Blake had found diseased larvae the year before. Some of the larvae were adhering to the food plants, although most of them were on the surface of the ground about the plants. Twelve of these were cultured, and each yielded gray colonies of an actively motile bacillus. A culture was made from a gray colony from each of the 12 plates. Inoculation of silkworms with these cultures showed 11 to be pathogenic, indicating a disease of the septicemia group. It seemed important that the culture from one gray colony was not pathogenic.

On August 20, 1923, a search was made for potato beetle larvae on the potato plants of the plot in Washington where, during each of the 2 preceding years, diseased larvae had been collected. Only six small to medium-sized larvae were found. Three of these were dead, but the other three presented no noticeable symptoms of disease. Agar plates made from each of the six larvae yielded gray colonies in each instance. Five silkworms were inoculated from each plate, a different colony being used for each worm. All the 30 worms died on the following day, the remains in each instance soon becoming soft and brown. These observations indicated that the 3 dead larvae had died of potato beetle septicemia and that the other 3, while not yet showing symptoms of the disease, were infected.

No dead potato beetle larvae were found during 1924 on the potato plot where during each of the 3 preceding years the disease had been encountered. Death from the disease may have occurred, however, since dead larvae may easily pass unnoticed on soil which has recently been cultivated and which has not been smoothed and hardened by rains.

The potato plot in Washington which offered the opportunity for a study of the occurrence of potato beetle septicemia and furnished much of the material for these studies had been used for potatoes for a number of years. As the primary purpose in maintaining the plot was to provide material for biological studies on the Colorado potato beetle, at least two plantings were made in it each year. After 1924 the plot was used for another purpose.

VIRULENCE OF *BACILLUS LEPTINOTARSAE*

Other data regarding the virulence of *Bacillus leptinotarsae* are shown in table 1, which gives the results with 15 cultures, each isolated from a different larva found in nature sick or dead of potato beetle septicemia.

The results recorded in table 1 may be summarized as follows: Of 88 potato beetle larvae inoculated, 85 died within 2 days; of 244 silkworm larvae, 243 died within 1 day; all of 15 hornworm larvae died within 2 days; all of 17 cutworm larvae died within 3 days; all of 19 catalpa sphinx larvae died within 2 days; all of 4 white grubs died within 2 days; and of 19 adult potato beetles inoculated, 15 died within 11 days.

It will be observed that among the larvae inoculated by puncture with *Bacillus leptinotarsae* an extremely high mortality resulted within 3 days. Adults are not so susceptible to infection with this organism. It will be noted further that the virulence of cultures on artificial media is retained over long periods.

Feeding and immersion methods were also employed in attempts to transmit the disease to potato beetle larvae. In two tests in which 42 larvae were inoculated by feeding, 7 died within 8 days.⁸ Fifty larvae were placed on potted potato plants which had been sprayed with a 48-hour bouillon culture of the bacillus. No evidence of infection was seen among these. Of 75 larvae immersed in an aqueous suspension of an agar culture and then placed on a potted potato plant, 3 died within 5 days. Whether these 3 died from the inoculation is not known.

EXCITING CAUSE

Bacillus leptinotarsae is the bacillus found in potato beetle septicemia in nature and the one present in the septicemia experimentally produced in the insects inoculated by puncture with material from the

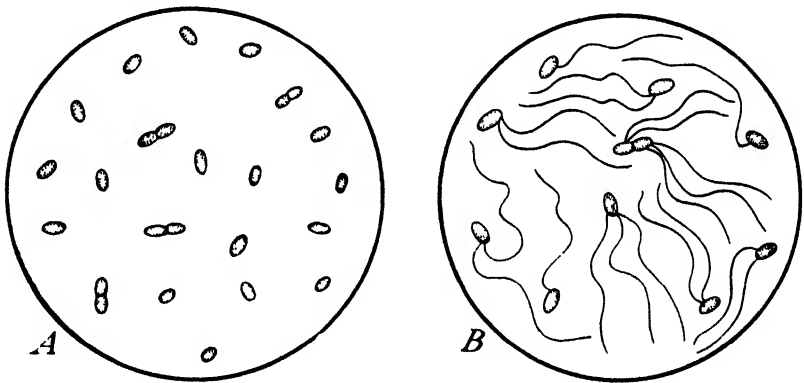


FIGURE 2—A, *Bacillus leptinotarsae*, B, flagella of *B. leptinotarsae*

potato beetle disease. Both in the disease as it occurs in nature and in the experimental one this bacillus seems to be the immediate cause of death. Whether it is the primary exciting cause of the potato beetle disorder is yet to be determined.

Inoculation of the common and usual differential media with *Bacillus leptinotarsae* is followed by abundant growth.

MORPHOLOGY.—The common name "coccobacillus" suggests the morphology of the species. The rods from a 1-day agar culture are small and short with rounded ends (fig. 2, A). Some are ovoid, and many resemble cocci. They measure about 0.9μ in length and 0.6μ in width. The rods from a 1-day bouillon culture measure 1.1μ in length. In older cultures they average slightly longer. Flagella are peritrichic (fig. 2, B); frequently only 1 is seen, and it is not polar. More often there are 2, 3, or 4, but rarely more than 4. Spores are not produced.

MOTILITY.—Many of the shorter rods progress actively.

STAINING PROPERTIES.—The rods stain readily and uniformly. They are Gram-negative.

AGAR PLATES.—Growth takes place rapidly. The colonies are gray by reflected and bluish by transmitted light. The border is entire and the surface oval and

⁸ Three of the 7 dead larvae were parasitized by fly larvae. The gross appearance of the others did not indicate that they had died from infection with *B. leptinotarsae*.

glistening. Magnified, they are finely granular. The mass is nonviscid and adheres to the medium.

AGAR SLANT.—Within 1 day a moderate bluish-gray nonviscid growth takes place.

GELATIN STAB.—A white growth with beginning of liquefaction is present along the stab after 24 hours at room temperature. The liquefaction continues and becomes crateriform, infundibuliform, and sometimes stratiform in 5 days. A friable pellicle forms which later sinks.

POTATO.—A moderate moist gray growth occurs within 2 days, which may become slightly brownish.

BOUILLON.—Growth takes place rapidly, the medium becoming slightly clouded within a few hours and heavily clouded within a day. A ring of gray growth adheres to the wall of the tube. A friable pellicle may form and later sink. The medium remains clouded and the sediment becomes heavy.

MILK.—A soft coagulum forms after 2 or 3 days and slow digestion takes place.

LITMUS MILK.—The milk is unchanged during the first day. Within a week the color is discharged.

CARBOHYDRATES.—Growth is usually increased by the addition of carbohydrates. Fermentation with acid production takes place in dextrose, galactose, levulose, maltose, mannose, mannite, glycerin, dextrin, xylose, and the glucoside salicin. The slight acidity occurring in lactose and arabinose is soon changed to alkaline. No acid is formed in raffinose, inulin, erythrite, or inosite.

THERMAL DEATH POINT.—A suspension in physiological salt solution is killed within 10 minutes at 52° C.

RESISTANCE TO DRYING.—An aqueous suspension from a 4-day agar culture on drying is dead within 3 hours.

PATHOGENESIS

Practically 100 percent of the potato beetle larvae inoculated by puncture with pure cultures of *Bacillus leptinotarsae* die from the infection produced. The period from inoculation to death ranges from less than 1 to more than 3 days. The course of the experimental disease varies with the insect species inoculated, the temperature, and slightly with the culture employed. Some cultures kept on agar with occasional transfers have retained their virulence for silkworms for more than 8 years. That of others has diminished.

A white mouse receiving intraperitoneally about 100,000 bacilli from a 48-hour agar culture became ill within 2 hours, later lying on its belly with the flanks depressed. Within a few hours it recovered and remained well thereafter. A rabbit similarly inoculated with about 200,000 bacilli was somewhat sluggish 4 hours after the inoculation, but soon recovered and remained well. A guinea pig, white rat, and two chickens receiving intraperitoneally about 200,000 bacilli manifested no symptoms of disease.

The recovery of *Bacillus leptinotarsae* from potato beetle larvae sick or recently dead of potato beetle septicemia, the experimental infection of healthy potato beetle larvae with a pure culture of the species by the puncture method, followed by death of the larvae with post-mortem changes similar to those of potato beetle septicemia, and the recovery of the bacillus in pure culture from the tissues of the experimental insect are easily accomplished. These observations tend strongly to indicate that the bacillus is the inciting cause of potato beetle septicemia. However, the extremely low mortality that follows both the feeding and the immersion methods of inoculation lead one to inquire further regarding the primary exciting cause of the disorder.

CULTURES FROM APPARENTLY HEALTHY LARVAE

Examinations were made of apparently healthy potato beetle larvae to determine the presence or absence of pathogenic bacterial species in the intestines. A bit of feces expressed from a larva and streaked on the surface of an agar plate usually sufficed for obtaining fairly well isolated colonies. One plate was made from each larva examined. Silkworms were inoculated by puncture from these plates, each worm receiving a culture from a different colony. Ordinarily five worms were inoculated from each plate. When the inoculated worms manifested symptoms and died, and the post-mortem changes resembled those of worms that had died from inoculation with *Bacillus leptinotarsae*, this pathogenic species was suspected.

The apparently healthy larvae, studied during 1923, were taken from potato plants grown on the same plot of ground where larvae, dead of potato beetle septicemia, had been found during each of the 2 preceding years. Gray colonies resembling those of *Bacillus leptinotarsae* were selected for examination.

On June 8, silkworms were inoculated from 3 plates. None of the 15 worms inoculated from these died. On June 9, silkworms were inoculated from 7 other plates. All the 25 worms inoculated from 5 of these plates remained well. All of the 10 worms inoculated from the other 2 plates died. On June 11, silkworms were inoculated from 12 plates. None of the 55 larvae inoculated from 11 of these plates died. Two of the five worms inoculated from the other plate died, the remaining 3 being unaffected.

The other observations for 1923 on apparently healthy potato beetle larvae are as follows: All the 10 gray colonies examined from 2 of 35 larvae were pathogenic to silkworms; 3 of the 5 colonies examined from another larva were pathogenic; and none of the colonies selected from plates made from the remaining 32 larvae were pathogenic.

The bacterial species isolated from apparently healthy potato beetle larvae which formed gray colonies and which was pathogenic to silkworms was found upon cultural study to be *Bacillus leptinotarsae*, the others were not.

Further studies were made during 1924 on the bacteria of apparently healthy potato beetle larvae. A species producing yellow colonies, one producing gray colonies similar to those of *Bacillus leptinotarsae*, and a species of *Streptococcus* were examined in addition to *Bacillus leptinotarsae*. Most of the larvae studied were taken from the same potato plot from which the larvae examined in 1923 had been collected.

On June 18, 10 agar plates were made, each with a bit of feces from a different potato beetle larva, and the gray colonies were chosen for testing. All of the 20 worms inoculated from 4 of the plates died, 14 of the 20 worms inoculated from 4 other plates died, and all of the 10 worms inoculated from the remaining 2 plates lived.

On July 15, 11 agar plates were made from 11 apparently healthy potato beetle larvae obtained from a locality 8 miles distant from the plot in Washington which had furnished larvae for the studies reported above. When 4 gray colonies from each plate were used, none of the 44 silkworms inoculated from the 11 plates died.

On June 25, 14 agar plates were made from 14 healthy larvae. In each instance the expressed feces were added to 1 cc of sterile water, and a plate was made from the suspension. Gray colonies were obtained on 10 of the 14 plates, yellow ones on 12 of them, and a species of *Streptococcus* on 6 of them. The number of yellow colonies per plate ranged from 1 to 14; the gray ones, from 1 to 300; and the *Streptococcus*, from 2 to 200, the larger counts being estimated in each instance.

On September 2, 16 agar plates were inoculated each with the diluted feces from a different potato beetle larva. One of the plates remained sterile. The rest bore gray colonies ranging in number from a few to many. Some of them also contained yellow colonies of a nonpathogenic, nonmotile bacillus and a few contained a species of *Streptococcus*. The pathogenic species forming gray colonies studied during 1924 were found to have the cultural characteristics of *Bacillus leptinotarsae*.

From this preliminary study on apparently healthy potato beetle larvae it is seen that some larvae taken from a plot where potato beetle septicemia had occurred harbored *Bacillus leptinotarsae*, and that a nonpathogenic bacillus resembling *B. leptinotarsae*, a nonpathogenic streptococcus, and a nonmotile, nonpathogenic chromogen were often present also.

COMPARISON OF *BACILLUS LEPTINOTARSAE*, *B. SPHINGIDIS*, AND *B. NOCTUARUM*

The morphology and cultural characteristics of *Bacillus leptinotarsae*, *B. sphingidis*, and *B. noctuarum* are similar. Variations within each species are comparable to the slight variations noted for the different species. The pathogenesis of these different species observed when silkworms are inoculated by puncture is also similar. Differences have been found in agglutination tests for the different species. Agglutination variations, too, are found among the different cultures of *B. leptinotarsae* isolated from the same outbreak of the disease. Serum from a rabbit immunized with *B. leptinotarsae* showing an agglutination titer of 1 : 1,600 for the culture used agglutinated some other cultures of the species at 1 : 1,600, some at 1 : 400, some at 1 : 200, and some were not agglutinated.

In giving another name to the bacillus occurring in potato beetle septicemia which is so similar to the species occurring in hornworm septicemia and to the one in cutworm septicemia, the writer is following the example of others working on this disease group. When the group has been more fully studied a different classification of these species may be made.

DIAGNOSIS OF POTATO BEETLE SEPTICEMIA

The presence of potato beetle septicemia may be suspected if the number of larvae in a beetle infestation should diminish rapidly from no apparent cause. The disease is further indicated if dead larvae of different ages showing post-mortem changes characteristic of the disease are found on the ground. A positive diagnosis is made by finding *Bacillus leptinotarsae* in sick or dead larvae. The disease must be differentiated from insect parasitization and death from insecticides.

PREDISPOSING CAUSES OF THE DISEASE

If *Bacillus leptinotarsae* is the primary exciting cause of potato beetle septicemia, predisposing factors probably play an important role in the causation of the disease. Since few if any potato beetle larvae died from feeding or immersion inoculations, these insects, it seems, must possess protective agencies which tend to prevent the entry of the organism into the blood.

Most of the larvae found dead of the disease in nature were either small or of medium size. In experimental inoculations also larvae of these sizes died sooner than those that were nearly mature.

Death from inoculations takes place sooner during hot weather than when the temperature is lower. That the season of the year is a predisposing factor for the disease is indicated by the observations that the heaviest mortality occurs in August.

TRANSMISSION OF THE DISEASE

Since the intestinal tract, the blood, and various tissues of the potato beetle larvae are places of multiplication of *Bacillus leptinotarsae*, the feces of such larvae and the bodies of those dead of the disease may contaminate the soil with the bacillus. As the viability of the bacillus is well maintained in damp earth, the soil remains a possible source of infection for long periods.

While it seems likely that the bacillus may at times gain entrance to the blood of the larvae by way of the alimentary tract, experimental evidence indicates that the insect possesses protective forces which markedly restrict infection by this route. Owing to the ease with which infection may be induced by puncture inoculations, injuries to the body wall by enemies of the larvae are to be considered as possible factors in the transmission of the disease. Among enemies reported for the potato beetle are a ladybird, ground beetles, soldier bugs, robber flies, a tachinid fly, mites, and spiders.

BACILLUS LEPTINOTARSAE AS A FACTOR IN THE CONTROL OF THE COLORADO POTATO BEETLE

In each year of the three that potato beetle septicemia was found in the field the presence of the disease was accompanied and followed by a rapid decrease in the number of larvae in the infestation, although there were at the time an abundance of food and many adult beetles. In the absence of any known cause for the decrease the disease must be considered a possible causative factor.

The heaviest mortality accompanying the disease has been noted among larvae of the last brood for the year. If the overwintering insects should be largely of this brood the disease would seem to have an added importance in the natural control of the pest.

Inasmuch as the disease is not readily transmitted through feeding inoculations with *Bacillus leptinotarsae*, the spraying of potato plants with cultures of this organism cannot be recommended at the present time as a means of artificial control. Whether artificial contamination of the soil with disease material or some other artificial use of the disease would be helpful in effecting control are problems for further study.

More data on the distribution and seasonal incidence of, and mortality from, the disease are much needed. It would be particularly instructive to know what insect and other enemies of the potato beetle are operating before and during the occurrence of the disease. Such observations would furnish useful information regarding the causes of the disease, its importance in the natural control of the pest, and its possible use in artificial control measures.

SUMMARY AND CONCLUSIONS

The present paper includes a description of, and a report on, preliminary studies of a disease of the Colorado potato beetle.

A frank septicemia in the moribund insect is a marked characteristic of the infection. This fact suggested the name potato beetle septicemia for the disease.

The bacillus occurring in the septicemia is a short, actively motile, Gram-negative rod, to which has been given the name *Bacillus leptinotarsae*.

Another bacillus found in cultures from larvae dead of potato beetle septicemia is morphologically and culturally similar to *B. leptinotarsae*, but is not pathogenic.

A nonpathogenic streptococcus occurred in considerable numbers in some of the larvae sick or dead of the disease.

Plate cultures made from feces expressed from healthy potato beetle larvae usually yielded only a small number of colonies. Only a few species occurred, and these often were chromogens.

The virulence of cultures of *B. leptinotarsae* on artificial media has changed little in 8 years.

It seems probable that potato beetle septicemia is a factor in the natural control of the Colorado potato beetle. Further studies are needed to demonstrate its importance in artificial control measures.

ROSE BLAST INDUCED BY PHYTOMONAS SYRINGAE¹

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INTRODUCTION

Early in May 1933, a malady which apparently has not been described was detected in a rose garden at Fayetteville, Ark., on the well-known hybrid perpetual, *Magna Charta*. It involved receptacles, calyx lobes, flower stalks, and leaf petioles, being most abundant on receptacles and relatively rare on leaf petioles.

SYMPTOMS

The affected portions appeared as blackish-brown, dead spots and streaks, which varied considerably in size and shape (fig. 1). Some took the form of small oval-shaped or rounded spots, not more than 1 mm in diameter, while others were large narrow streaks involving 5 to 8 cm of tissue in the longest diameter. The individual spots and streaks appeared depressed or sunken as compared with adjoining healthy tissue, particularly on the receptacles and pedicels, and suggested a collapse of the cells involved in the diseased areas. A narrow red border often delimited the diseased parts. On the large calyx lobes, characteristic of *Magna Charta*, the disease, starting at the base, often ran the whole length of the lobe. In most instances the affected buds failed to open, and may be said to have been blasted or blighted.

Microscopic examinations of diseased areas revealed great numbers of bacteria within the tissues, the bacteria readily clouding the water medium when slight pressure was applied to the cover glass. In this respect the disease closely resembled fire blight on rose buds. Indeed, the external symptoms bear a very close resemblance to those pictured by Rosen and Groves² on rose buds artificially infected with *Erwinia amylovora*, except that droplets of bacterial exudate were not observed in the disease here discussed.

INOCULATION EXPERIMENTS

Several pure-culture isolations were attempted, and isolates were obtained from two diseased receptacles, a calyx lobe and a petiole. Special studies were made of the morphology, the cultural and physiological reactions, and the pathogenicity of these isolates. Since artificial inoculations on shoots of pear (*Pyrus communis*) (fig. 2) resulted in blackish discoloration of leaf blades that were

¹ Received for publication Mar. 27, 1935; issued September 1935. Research Paper No. 391, Journal Series, University of Kansas.

² ROSEN, H. R., and GROVES, A. B. STUDIES ON FIREBLIGHT: HOST RANGE. Jour. Agr. Research 37: 493-505, illus. 1928.

located considerably beyond the bacteria-invaded stem tissue, it appeared that the pathogen might belong to the *Phytomonas syringae* group and not to *Erwinia amylovora* (see Rosen and Bleeker).³ To test this theory the rose isolates were grown on Uschinsky's solution and on beef-infusion agar, two media which are conducive to the



FIGURE 1.—Natural infections on rose calyx lobes, receptacles, pedicels, and petiole (extreme left) of *Magna Charta*. Slightly reduced.

development of the greenish fluorescent pigment characteristic of *P. syringae*. All of the rose isolates, when transferred to these media, developed a pigment which appeared very similar to that produced by authentic cultures of *P. syringae*, *P. citriputeale*, and *P. prunicola*.

³ ROSEN, H. R., and BLEEKER, W. L. COMPARATIVE SEROLOGICAL AND PATHOLOGICAL INVESTIGATIONS OF THE FIRE-BLIGHT ORGANISM AND A PATHOGENIC FLUORESCENT GROUP OF BACTERIA. *Jour. Agr. Research* 46: 95-119, illus. 1933.

Former work by Rosen and Bleecker had shown the lemon to be a differential host between *Erwinia amylovora* and *Phytomonas syringae*, the fire blight pathogen producing little or no effect on this fruit while the various forms of *P. syringae* produced typical black pit lesions. Consequently, if the rose isolates were related to *P. syringae*, they should produce conspicuous blackish depressed spots on lemons.



FIGURE 2.—Artificial infections on Bartlett pear shoots with a pure-culture isolate from a diseased rose receptacle. Note blackish discolorations of stem tissues (around points of inoculation), and the sootylike discolorations of the extreme upper leaf blade, central shoot, characteristic of such artificial infections on pear, and serving to distinguish *Phytomonas syringae* from *Erwinia amylovora*.

Needle-prick inoculations on 12 lemons with these rose pathogens, contrasted with *E. amylovora* inoculations made at the same time, resulted in the production of blackish spots with the rose isolates and none with the fire blight pathogen (fig. 3). The spots were characteristic of those produced by *P. citriputeale* (one of the many names under which *P. syringae* has been described). For a relatively rapid

and simple differential test between *E. amylovora* and fluorescent rosaceous bacterial pathogens, the use of lemon fruit appears to offer exceptional advantages.

In addition to pear twigs and lemon fruit, artificial inoculations were made on two varieties of roses, Magna Charta and Radiance (pink), and on young lilac shoots. All the inoculations on Magna Charta were made out of doors in a garden which had shown no

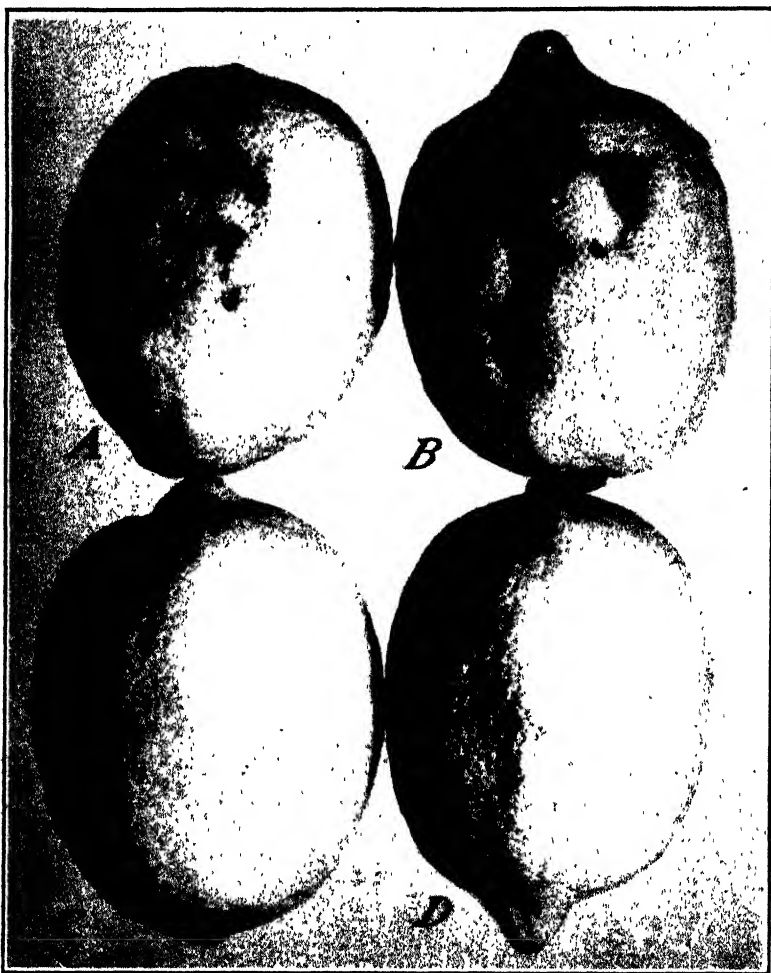


FIGURE 3.—Artificial needle-puncture inoculations with pure-culture isolates from a diseased rose receptacle (A), and a diseased leaf petiole (B); control, needle-puncture injections with sterile water (C), and with *Erwinia amylovora* (D). Note marked infections with the rose isolates, and the little or no effect of *E. amylovora*.

signs of the disease. On April 29, 1934, 11 flower buds of this variety were inoculated by means of needle punctures comparable to wounds made by thorn injuries, the inoculum consisting of 4-day-old nutrient dextrose-agar slant cultures of a rose-receptacle isolate suspended in sterile distilled water. Several leaf and flower clusters were atomized with similar water suspensions without wounding, the

bacteria being applied by means of sterilized, all-metallic artists' atomizers. These atomizer inoculations were enclosed in glassine bags. A similar number of control inoculations were made by means of needle punctures and the use of sterile water, and by atomizing unwounded shoots with sterile water and covering them with glassine bags. On May 3 all needle-puncture inoculations with bacteria showed symptoms resembling natural infections of rose blast (fig. 4). All the controls were free from disease symptoms other than very limited brownish spots centering around the needle pricks. By May 8 the infected areas on some of the buds inoculated by needle punctures had involved one-third or more of the receptacle circumference, 1 or 2 calyx lobes, and the upper third of the pedicels. In these inoculations, the portion of the bud which showed no depressed, brownish areas turned a sickly yellow and the buds with attached pedicels aborted and dropped from the plant. All checks remained healthy, the blossoms opening normally and remaining attached to the plant. No sure signs of infection were obtained in the unwounded atomizer inoculations. From one of the artificially infected buds the organism was reisolated, grown in pure cultures, and inoculated on several Bartlett pear shoots, on which typical *Phytophthora syringae* symptoms appeared.

Numerous attempts to infect Radiance plants maintained in a greenhouse by needle-puncture inoculations, by hypodermic injections, and by spraying with bacterial water suspensions on young buds and leaves, were failures. Beyond wound injuries made by the needles, the inoculated plants showed no signs of disease. Under greenhouse conditions at least, Radiance appears to be nonsusceptible to this disease when Bartlett pear shoots similarly inoculated are very susceptible.

On April 5, 1934, tender shoots of lilac (*Syringa vulgaris*) attached to plants growing out of doors were inoculated with different isolates of *Phytophthora syringae* by means of hypodermic injections. The first signs of infection appeared on April 9. Ten shoots were inoculated with each isolate. The number infected is shown below.

Isolate:	Number of shoots infected
Rose receptacle.....	8
Pear receptacle ⁴	7
Pear petal ⁴	7
<i>P. syringae</i> from lilac ⁴	1
<i>P. prunicola</i> ⁴	0
Pear (Garber) ⁴	0
Controls (sterile water).....	0

In addition to the hypodermic injections, several lilac leaf clusters were inoculated without wounding by atomizing with water suspensions of the following isolates: Rose receptacle, pear petal, and *Phytophthora syringae*. Six days later the only lilac leaves infected were those atomized with the isolate from rose receptacle, and even these were very small, pin-point lesions on a few leaves. These results coupled with those shown above, leave no doubt that the rose isolate is parasitic on lilac (fig. 5). The fact that the rose isolate

⁴ These isolates were the ones previously studied by Rosen and Bleecker. See footnote 3.



FIGURE 4.—(A, B), Artificial needle-puncture inoculations on Magna Charta rose with a pure-culture isolate from rose blast; C, needle puncture on control. Photographed 9 days after inoculating. Compare with figure 1.

produced a greater number of infections on this host than the lilac blight isolate can perhaps be explained by the fact that the latter had been kept growing in artificial cultures for several years and had



FIGURE 5.—Artificial infections on lilac with a pure-culture isolate from rose blast. Photographed 5 days after inoculating.

probably lost most of its virulence. The same explanation may be offered for lack of infections with several of the other isolates. The stem lesions produced by the rose blast isolates on lilac are comparable to those described by Bryan ⁵ for lilac blight.

⁵ BRYAN, M. K. LILAC BLIGHT IN THE UNITED STATES. Jour. Agr. Research 36: 225-235, illus. 1928

CULTURAL CHARACTERS OF THE ORGANISM

In culture media and in stained preparations, the rose blast isolates are comparable to the other isolates noted previously. They are all rapidly growing, motile, polar-flagellated rods (fig. 6), which as isolated colonies on nutrient agar plates are round, glistening, white or grayish white, butyrous, pulvinate, and definite-margined. If beef-infusion agar is used instead of beef extract, the agar around the colonies turns greenish yellow. Comparable pigment is produced in Uschinsky's solution and in beef-infusion broth.



FIGURE 6.—Pure-culture isolate of a rose receptacle, from a 20-hour-old nutrient agar slant culture grown at 26° C.; stained to show flagella (Rosen Cr 1 M stain). Note polar arrangement. $\times 1,425$.

COMPARISON OF ROSE BLAST WITH OTHER ROSE DISEASES RESEMBLING IT

As to other diseases of roses which resemble rose blast or which may be confused with it, the bacterial disease recently described very briefly by White ⁶ is of special interest. He noted it on greenhouse roses, particularly on the hybrid tea varieties Souvenir and Talisman. It appeared as brownish spots, often with a reddish border on leaves (blades?), on calyxes, on outer petals (in the form of "dark brown water-soaked decay"), and on young stems (small, reddish spots). He briefly notes that the bacterial colonies are gray (yellowish by transmitted light, as many grayish or whitish bacteria are likely to be), "circular with smooth regular margins and a glistening surface." So far as this description of the bacteria is concerned, it might be applied to *Phytomonas syringae*, though it is entirely too inadequate to be applied with certainty. Nothing is said about any pigment production on various culture media, and some of the symptoms as

⁶[WHITE, R. P.] A BACTERIAL DISEASE OF ROSE. N. J. Agr. Expt. Sta. Nursery Disease Notes. v. 5 (5): 1-2, 1932. [Mimeographed.]

well as the conditions under which the disease is produced suggest that it is different from rose blast.

Several other maladies which may be confused with bacterial blast of roses are of nonparasitic origin. These are (1) a browning and withering of flower buds of *Rosa rugosa* varieties and nearly all hybrid rugosas grown in the South, including F. J. Grootendorst and Sir Thomas Lipton. This the writer considers to be in the nature of species or varietal response in regions unadapted or adverse to this particular group of roses. (2) The well-known browning and blighting of many hybrid perpetuals and nearly all hybrid teas and teas which possess many petals, a malady which is seemingly associated with extremes of temperature, with perhaps the low temperatures doing most of the damage. In the South, General Jacqueminot, a great favorite of northern rose growers, is notoriously susceptible to this form of disease, while among hybrid teas Francis Scott Key, Dame Edith Helen, Jonkheer J. L. Mock, Willowmere, and Columbia not infrequently develop a "balling" and blighting of flower buds, particularly in the early part of the growing season. Various types of insect injury at times involve a browning and killing of the buds.

DISTRIBUTION OF ROSE BLAST AND OBSERVATIONS ON VARIETAL BEHAVIOR

The fact that rose blast is here reported apparently for the first time and from one locality only, suggests that it is limited in range or that it is of relatively rare occurrence. However, in view of the wide range of hosts that have been described for various isolates of *Phytomonas syringae*, if the writer's identification of the pathogen is correct it may be widely distributed, both in this country and in Europe, but limited in occurrence by weather conditions. It is to be looked for primarily in the early part of the growing season during exceptionally cool and wet weather.

As to varieties other than Magna Charta that may be susceptible to rose blast, Georg Arends, a hybrid perpetual, growing in another rose garden at Fayetteville, had flower buds that appeared to be affected with this disease. The diagnosis was uncertain, however, no culture work having been attempted. It is of interest to note that standing close to the diseased Magna Charta were a number of other rose varieties, including at least three hybrid perpetuals, Paul Neyron, Mrs. John Laing, and Frau Karl Druschki; several hybrid teas and teas, including Red-Letter Day, Madam Butterfly, Talisman, Red Radiance, Dame Edith Helen, and White Maman Cochet; and a supposed polyantha, Gruss an Aachen, which showed no symptoms of the disease. Whether this is indicative of a true resistance of these roses or whether it was merely a fortuitous occurrence cannot be stated with certainty.

SUMMARY

A disease of roses involving receptacles, calyx lobes, pedicels, and petioles is described. The disease is believed to be new to science. The malady termed "rose blast" is caused by bacteria, and the pathogen has been identified as *Phytomonas syringae*.

INHERITANCE OF RESISTANCE TO MILDEW, ERYSIPE GRAMINIS HORDEI, IN A CROSS BETWEEN HANNA AND ATLAS BARLEY¹

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INTRODUCTION

Barley mildew (*Erysiphe graminis hordei*) is a common disease in California. Although usually present in fields of barley (*Hordeum Vulgare* L.), it is especially destructive to late-sown barley. Since the production of resistant varieties offers the only practical control, a study of the inheritance of resistance to mildew in hybrids between several resistant varieties and Atlas barley, which is very susceptible has been undertaken. As barley offers very good material for link, age studies, an attempt is being made to place the resistant factors in their proper linkage groups. The genetic information obtained will form the basis for a program of backcrossing by which it is expected to transfer resistance to the most important of the barley varieties grown in California. The present paper deals with data from the hybrids of Hanna \times Atlas.

WORK OF OTHER INVESTIGATORS

As Hansen (7)³ has recently published an extensive review of the literature dealing with the inheritance of resistance to plant diseases, only a few papers will be mentioned here.

Biffen (1), as early as 1907, studied the inheritance of resistance to mildew in a cross between *Hordeum spontaneum* and *H. hexastichocorym*, the former being the most resistant and the latter the most susceptible variety in a collection of 140 varieties. The F₁ progeny was as completely attacked as the susceptible parent. In an F₂ population of 79 plants 56 were badly attacked, 16 bore a trace of the mildew, and 7 were free from it. If in addition to the 7 mildew-free plants the 16 that had a trace of the disease are considered as resistant—a conclusion which seems justified since *H. spontaneum* showed a trace of the disease under the same conditions—the result is 56 susceptible to 23 resistant. Although the number of plants was small and the results were not verified in the F₃ generation the data suggest the operation of a single recessive factor for resistance to mildew.

Dietz and Murphy (4) and Dietz (3) studied the inheritance of resistance to mildew in crosses between resistant Goldfoil C. I. 928 and four susceptible barley varieties, Chevalier C. I. 156, Trebi C. I. 936, Odessa C. I. 927, and Velvet C. I. 4252. In the four crosses

¹ Received for publication Apr. 12, 1935; issued September 1935.

² The writer wishes to acknowledge the assistance of L. D. Whitney and G. L. Barry in taking the mildew readings.

³ Reference is made by number (italic) to Literature Cited, p. 249.

there were 588 susceptible and 202 resistant plants—very close to a 3:1 ratio. Taken individually, all the crosses gave ratios very close to 3:1 except Goldfoil \times Velvet. Only 48 F_2 plants, however, were grown from that cross. Enough F_2 progenies were grown to verify the F_2 classifications. The factor for resistance to mildew in the Goldfoil crosses differs from that in the Hanna hybrids, because in the former susceptibility is completely dominant, as Biffen found it in *Hordeum spontaneum*, whereas, as will later be shown, susceptibility is incompletely dominant in the Hanna cross.

MATERIALS AND METHODS

In the fall of 1929 Dr. E. B. Mains, then at Purdue University, generously supplied the writer with several barley varieties known by him to be resistant to one or more physiologic forms of barley mildew. These were selected from varieties used by Mains and Dietz (8) in studying physiologic forms of this disease. Of the 5 forms discovered by them, Hanna C. 1. 906 was completely resistant to 4 but susceptible to the fifth, giving a type 4 infection. They recognized 5 types of reaction, 0 to 4, as follows (8, p. 231):

Highly resistant, denoted as 0. Macroscopically, no mycelium is evident. Chlorotic or necrotic spots may be developed by some varieties. Microscopically, a slight amount of mycelium may develop and infection may be evident macroscopically by faint flecks.

Very resistant, denoted as 1. Slight to moderate development of mycelium evident macroscopically but with little or no sporulation. Chlorotic or necrotic spots developed by some varieties.

Moderately resistant, denoted by 2. A moderate to abundant development of mycelium occurs, accompanied by a slight production of conidia. Chlorotic or necrotic areas are formed by some varieties.

Moderately susceptible, denoted by 3. A moderate to abundant development of mycelium occurs, accompanied by moderate sporulation.

Very susceptible, denoted by 4. Abundant mycelium is developed, accompanied by abundant sporulation.

The writer has followed this classification in designating the reaction of the varieties which were supplied by Mains, and which have been grown in the field at Davis, Calif., since 1930. As the 1930 crop was seeded in the fall, comparatively little mildew developed. Since that time these barleys have been seeded in late spring, and good mildew infections have resulted, although the readings have not always been so high as those obtained by Mains and Dietz in their greenhouse studies. The mildew here has corresponded very closely to their form 3, the only difference being a reading of 1 on an unnamed variety, C. I. 1021, where they obtained 0. It was interesting to find form 3, since their form 4 originally was collected in California and Idaho. According to Dietz (3), Dr. Lysle D. Leach collected form 4 at Davis, Calif., in 1927, 1928, and 1929.

The hybrid populations were grown in the field at Davis in the same nursery with the differential host varieties and were seeded at the same time. They were grown in rod rows 1 foot apart, the F_2 seed being spaced 6 inches part and the F_1 4 inches. Every fifth row was seeded to Atlas as a check. The disease developed entirely from natural infection. The mildew readings were made at about the time the plants were heading.

EXPERIMENTAL RESULTS

CLASSIFICATION OF F_2 AND F_3 PLANTS

From 1930 to 1934, Hanna was given a reading of 0. On a few occasions some mycelium developed, but in every case it was limited to 1 or 2 spots on an entire plant. Under the same conditions Atlas invariably was given a reading of 4.

The F_2 plants were grown in 1933. They were classified as either susceptible or resistant. Every plant on which any mildew was found was classed as susceptible. The resistant plants were tagged so that the classification could be checked in F_3 . Out of a total of 386 F_2 plants, 286 were susceptible and 100 resistant. This is very close to a 3:1 ratio, with 289.5 and 96.5 the numbers expected.

Considerable variation was noted in the development of mildew on the susceptible plants, ranging from type 1 to type 4. It is believed that the lower grades of infection represent heterozygous plants, that type 4 and possibly type 3 represent homozygous susceptible plants, and that the F_2 plants could have been classified into a 1:2:1 ratio. This observation was confirmed in a study of heterozygous F_3 rows which will be discussed presently.

The F_2 data indicate, therefore, that Hanna differs from Atlas in one factor for resistance to mildew and that susceptibility is incompletely dominant.

F_3 progenies of 30 to 40 plants were grown from 384 of the 386 F_2 plants. Two plants failed to produce seed enough for an F_3 row. The F_3 results are found in table 1.

TABLE 1.—Distribution of parent and F_3 rows of the cross named into susceptible, segregating, and resistant classes, grown in the field at Davis, Calif., 1934

Parent or hybrid	Rows of plants		
	Susceptible	Segregating	Resistant
	Number	Number	Number
Hanna parent.....			15
Atlas parent.....	101		
Hanna \times Atlas.....	97	193	94

The F_3 classification is very close to a 1:2:1 ratio, thus verifying the conclusion that the resistance of Hanna results from a single genetic factor. There had been a few errors in classifying the F_2 plants. In 5 cases plants marked resistant proved to be heterozygous, and 5 classified as susceptible proved resistant. This is not surprising, since even Hanna occasionally showed a trace of mildew and since in classifying the F_2 all plants showing any mycelial development were placed in the susceptible group. All homozygous susceptible plants had been correctly classified.

The segregating F_3 rows had an average of 73.9 percent susceptible plants. In the discussion of the F_2 results it was pointed out that not all the susceptible plants showed a high grade of infection, and it was suggested that heterozygous plants were intermediate in susceptibility. With this in mind a few segregating F_3 rows were classified as completely susceptible, intermediate, and resistant. The

results gave a satisfactory 1:2:1 ratio, which it has not seemed necessary to verify by growing a progeny test.

The F_3 data confirm the conclusion that Hanna differs from Atlas in one factor for resistance to form 3 of barley mildew, and that susceptibility is incompletely dominant.

LINKAGE STUDIES

In an extensive review of the literature on the inheritance of resistance to plant diseases Hansen (7) found only a few cases of linkage between disease resistance and morphological characters in plants. Griffiee (6), studying resistance to *Helminthosporium sativum* in relation to several characters in barley, found a positive correlation between resistance versus susceptibility and the factor pairs (1) six-rowed versus non six-rowed, (2) rough versus smooth awns, and (3) white versus black glumes. Because homozygous resistant progenies are not easily recognized, Griffiee (6) was unable to learn much about the genetics of resistance to this disease except that it was correlated with the three factor pairs mentioned above. Since these factor pairs represent 3 linkage groups, he concluded that at least 3 factors for resistance were present in the resistant variety Svanhals.

Barley offers favorable material for linkage studies because it has only seven pairs of chromosomes and the linkage between a number of factors has already been determined. Daane (2), in a critical survey of the literature on barley linkage, recognizes five well-established linkage groups with a number of factor pairs represented in each group.

In the present paper data are available on three pairs of factors: (1) Resistance versus susceptibility to barley mildew, (2) six-rowed versus non 6-rowed spikes, and (3) short-haired versus long-haired rachilla. Hanna is resistant to mildew, is two-rowed (*H. distichon* type), and has a long-haired rachilla. Atlas has the allelomorph to each of these characters. The two last-named factor pairs have been found by one or more investigators to give monohybrid ratios. That they segregated according to expectation may be seen in table 2.

TABLE 2.— F_2 segregation of the factors listed in the cross of Hanna \times Atlas, grown at Davis, Calif.

Character	Observed	Expected	χ^2	P ¹
	Number	Number		
Susceptible to mildew.....	290	288	0.0139
Resistant to mildew.....	94	96	.0417
Total.....	384	384	.0556	>.8
Non 6-rowed.....	279	288	.2813
6-rowed.....	105	96	.8438
Total.....	384	384	1.1251	>.3
Long-haired rachilla.....	283	288	.0668
Short-haired rachilla.....	101	96	.2604
Total.....	384	384	.3472	>.6

¹ Values of P taken from Fisher (5).

Daane (2) place nonsix-rowed versus six-rowed in linkage group 1 and long-haired versus short-haired rachilla in group 2. That they belong to different linkage groups is confirmed by data in table 3.

TABLE 3.—*F₂ data on independent inheritance of the factor pairs listed in the cross Hanna × Atlas, grown at Davis, Calif.*

Character	Observed	Expected	χ^2	P
	<i>Number</i>	<i>Number</i>		
Non 6-rowed, long-haired rachilla.....	211	216	0.1157	
Non 6-rowed, short-haired rachilla.....	68	72	.2222	
6-rowed, long-haired rachilla.....	72	72	.0000	
6-rowed, short-haired rachilla.....	33	24	3.3750	
Total.....	384	384	3.7129	>0.2

The data available from Hanna × Atlas, therefore, provide a test for linkage between mildew resistance and one character each in linkage groups 1 and 2. That independent assortment occurred is shown by table 4.

TABLE 4.—*F₂ data on independent inheritance of mildew resistance vs. susceptibility with the factor pairs listed; plants grown at Davis, Calif.*

Character	Observed	Expected	χ^2	P
	<i>Number</i>	<i>Number</i>		
Susceptible, non 6-rowed.....	208	216	0.2963	
Susceptible, 6-rowed.....	82	72	1.3889	
Resistant, non 6-rowed.....	71	72	.0139	
Resistant, 6-rowed.....	23	24	.0417	
Total.....	384	384	1.7408	>.5
Susceptible, long-haired rachilla.....	218	216	.0185	
Susceptible, short-haired rachilla.....	72	72	.0000	
Resistant, long-haired rachilla.....	65	72	.6807	
Resistant, short-haired rachilla.....	29	24	1.0417	
Total.....	384	384	1.7400	>.5

Since no linkage was observed between mildew resistance and the characters studied in linkage groups 1 and 2, crosses are being made which involve character pairs in other linkage groups. The writer hopes to place this disease-resistant factor definitely in one of the linkage groups.

SUMMARY AND CONCLUSIONS

A study was made of the inheritance of resistance to mildew in a cross between Hanna and Atlas barley.

The results show that Hanna differs from Atlas in one factor for resistance to mildew and that susceptibility is incompletely dominant.

There was no indication of linkage between mildew resistance and one factor pair each belonging to linkage groups 1 and 2 respectively.

LITERATURE CITED

- (1) BIFFEN, R. H.
1907-12. STUDIES IN THE INHERITANCE OF DISEASE-RESISTANCE. Jour. Agr. Sci. [England] 2:[109]-128, 1907; 4:[421]-429, 1912.
- (2) DAANE, A.
1931. LINKAGE RELATIONS IN BARLEY. Minn. Agr. Expt. Sta. Tech. Bull. 78, 30 pp.
- (3) DIETZ, S. M.
1930. THE VARIETAL RESPONSE AND INHERITANCE OF RESISTANCE IN BARLEY TO ERYSIPIHE GRAMINIS HORDEI. P. F. 4. Iowa State Col. Jour. Sci. 5:25-33, illus.

-
- (4) DEITZ, S. M., and MURPHY, H. C.
1930. INHERITANCE OF RESISTANCE TO ERYSHIPHE GRAMINIS HORDEI, p. f. iv. (Abstract) *Phytopathology* 20: 119-120.
- (5) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.
- (6) GRIFFEE, F.
1925. CORRELATED INHERITANCE OF BOTANICAL CHARACTERS IN BARLEY, AND MANNER OF REACTION TO HELMINTHOSPORIUM SATIVUM. *Jour. Agr. Research* 30: 915-935, illus.
- (7) HANSEN, H. P.
1934. INHERITANCE OF RESISTANCE TO PLANT DISEASES CAUSED BY FUNGI, BACTERIA, AND VIRA. A COLLECTIVE REVIEW WITH A BIBLIOGRAPHY. *K. Vet. og Landbohøjskole Aarsskr.* 1934: 1-74.
- (8) MAINS, E. B., and DIETZ, S. M.
1930. PHYSIOLOGIC FORMS OF BARLEY MILDEW, ERYSHIPHE GRAMINIS HORDEI MARCHAL. *Phytopathology* 20: 229-239, illus.

EFFECT OF THE STAGE OF MATURITY AND METHOD OF CURING UPON THE VITAMIN B AND VITAMIN G CONTENT OF ALFALFA, CLOVER, AND TIMOTHY HAYS¹

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INTRODUCTION

In the past hays have been produced largely with one object in mind—that of obtaining the utmost in quantity or total yield. More recently attention has been directed to the factors that affect the nutritive properties of hays, with the object of producing a product with the maximum feeding value.

Many investigators have pointed out the importance of hays as a source of vitamins for growing and producing animals. These investigations have in the main dealt with vitamins A and D. Practically no information is available regarding the vitamin G content of hays and the factors that affect it. This report presents the results of a study of the vitamin B (B_1) and G (B_2) content of alfalfa, clover, and timothy hays as they are influenced by the stage of maturity of the plant when cut and by natural climatic factors. It also includes the results of growth experiments with chicks, designed to show the value of the chick method of assay for the vitamin G complex as compared with the rat method.

REVIEW OF LITERATURE

In reviewing the literature, one finds data showing that certain practices or procedures affect the vitamin content of hays. Steenbock, Hart, and their associates (10)² found that clover hay which had been exposed to sunlight and rain was less valuable as a source of vitamin A and that it had better calcifying properties than hay which had been cured quickly and still retained a good green color. Russell (9) confirmed these findings, by showing that alfalfa hay dried artificially was about seven times as potent in vitamin A as hay cured in the usual way. He also noted an increase in the vitamin D content when hay was cured in the sun. Unpublished data obtained at the Ohio Station confirm the foregoing results. Bethke and Kick (1) observed a loss of vitamin A in alfalfa hay cured in the sun and exposed to rain and dew. Hauge and Aitkenhead (7) also observed a loss of vitamin A and explained that the destruction was due to enzymes. Hathaway, Davis, and Graves (6) found that artificially cured alfalfa was twice as potent in vitamin A as field-cured alfalfa. Douglass, Tobiska, and Vail (5) reported that alfalfa cut at the early bloom stage contained more vitamin A and vitamin G than hay cut at a later stage. They also reported that exposure to rain and sunshine lowered the vitamin A, vitamin B, and possibly the vitamin G

¹ Received for publication Feb. 9, 1935; issued September 1935.

² Reference is made by number (italic) to Literature Cited, p. 258.

content. Hunt and Krauss (8), in a study of pasture grasses, found that the vitamin G content decreased as the plants matured.

SOURCE OF HAYS

The alfalfa and clover hays used in this investigation were either grown on the station farm at Wooster or were obtained from the Department of Farm Crops of Ohio State University at Columbus. The timothy samples were grown on the station farm or on plots of the Timothy Breeding Station of the United States Department of Agriculture, at North Ridgeville, Ohio. Unless otherwise stated, the samples were cured indoors in subdued light.

RAT EXPERIMENTS

METHODS

In the first series of experiments for evaluating the vitamin content of the hays, rats were used as the experimental animals. These animals were born to mothers maintained on a modified Steenbock stock ration (2). When 24 days of age, or when weighing from 45 to 60 g, the young were placed in screen-bottom cages and were given a diet free from vitamin B and vitamin G. This diet consisted of casein (extracted if found to contain vitamin B or G), 18 percent; cornstarch, 64; hydrogenated fat (Crisco), 10; agar, 2; cod-liver oil, 2; and salt mixture (185), 4 percent.

If the rats were to be used for the determination of vitamin G each was given daily 400 mg of yeast (Northwestern), autoclaved for 4 hours at 15 pounds pressure, in addition to the basal diet. When the animals had ceased gaining in weight, which required from 14 to 21 days, they were placed in individual wire cages and fed daily the hay supplement with 400 mg of autoclaved yeast. The supplements were moistened with distilled water to prevent scattering.

If the rats were to be used for the determination of vitamin G, the basal diet was supplemented with 1 cc of an extract of rice polishings per rat daily, to furnish vitamin B. When the weights of the animals became stationary, or when they had lost slightly in weight, they were transferred to individual wire cages and the supplement (hay), moistened with 1 cc of the vitamin B preparation, was fed daily. The depletion period required on an average about 24 days.

The vitamin B extract was prepared as follows: Four kilograms of rice polishings were shaken up with 8 l of 0.1-percent hydrochloric acid and allowed to stand overnight. The clear supernatant liquid was then siphoned off. This procedure was repeated 5 or 6 times. The combined extracts were evaporated to 1,200 to 1,400 cc and then made up to 80 percent alcohol by weight. The precipitate was filtered off and washed with alcohol of the same strength. The combined alcoholic filtrates were partially concentrated under vacuum and the last traces of alcohol removed before an electric fan. The residual liquid was then diluted so that 1 cc was equivalent to 1 g of rice polishings.

The hays were ground to a fine flour, in a ball mill, for feeding purposes. The experimental feeding period in all cases was of 6 weeks' duration. The results are expressed in terms of rat units, as outlined by Bourquin and Sherman (3) and Chase and Sherman (4).

TABLE 1.—Vitamin B and vitamin G value of hays

VITAMIN B							Source and description of hay
Sample and no. ¹	Date cut	Rats	Level fed daily	Total gain or loss (—) in weight	Weekly gain or loss (—) in weight	Vitamin units per gram (approximate) ²	
		No.	Milli-grams	Grams	Grams		
Alfalfa (1)-----	May 31, 1932	{ 8	350	11.7	1.9	2.2	University farm; first cutting
	June 10, 1932	{ 8	450	22.3	3.7		
	June 21, 1932	{ 8	400	15.3	2.5		
	June 21, 1932	{ 8	500	18.1	3.0	1.6	Second cutting; injured by leaf hoppers.
	July 28, 1932	{ 8	500	8.1	1.5		
	Sept. 10, 1932	{ 8	600	18.3	3.0	2.0	Third cutting.
Alfalfa (2)-----	June 8, 1932	{ 8	450	13.3	2.2	2.0	Station farm.
	June 8, 1932	{ 8	500	18.2	3.0		
	June 12, 1931	{ 8	400	19.0	3.1	2.5	U. S. Timothy Breeding Station
	June 24, 1931	{ 8	500	26.0	4.3		
Timothy (3)-----	July 13, 1931	{ 8	600	7.2	1.2	1.7-0.8	U. S. Timothy Breeding Station; late maturing
	June 18, 1932	{ 8	700	5.1	.85	3.6	
	June 18, 1932	{ 8	900	21.8	3.6	3.1-2	
Timothy (3), 15150	do	{ 8	600	12.5	2.0	1.4	Regular cutting
Timothy (3), 12368	July 1, 1932	{ 8	800	23.8	3.9		
Clover (1)-----	June 10, 1932	{ 8	600	6.5	1.1	1.6	University farm.
	June 21, 1932	{ 8	1,000	11.5	1.9		
	June 21, 1932	{ 8	500	13.1	2.2		
Alfalfa from alfalfa and timothy mixture (2).	June 8, 1932	{ 8	600	22.3	3.7	1.6-1.8	Station farm, grown as a mixed hay
	do	{ 8	500	13.1	2.2		
	do	{ 8	600	27.2	4.5		
Alfalfa and timothy mixture (50:50) (2).	June 8, 1932	{ 8	400	6.6	1.1	1.6	Station farm, first cutting.
	do	{ 8	600	22.2	3.7		
	do	{ 8	600	11.4	1.9		
400 mg autoclaved yeast (controls).	do	{ 8	800	24.5	4.1	1.4-1.5	Second cutting, injured by leaf hoppers.
	do	{ 8	650	16.7	2.8		
	do	{ 8	700	21.3	3.5		

VITAMIN G

Alfalfa (1)-----	May 31, 1932	{ 8	100	25.1	4.2	10-12	University farm; first cutting
	June 10, 1932	{ 8	150	40.8	6.8		
	June 21, 1932	{ 8	100	24.9	4.1		
	June 21, 1932	{ 8	150	27.4	4.5	6.6	Second cutting; injured by leaf hoppers.
	July 28, 1932	{ 8	200	34.8	5.8		
	Sept. 10, 1932	{ 8	150	24.0	4.0	6.6	Third cutting.
Alfalfa (2)-----	June 8, 1932	{ 8	75	22.6	3.8	13.0	Station farm; first cutting.
	June 8, 1932	{ 8	100	15.9	2.6		
	June 8, 1932	{ 8	150	16.7	2.8		
	July 21, 1932	{ 8	100	18.5	3.0	10.0	Second cutting, sun-cured; no rain.
	July 21, 1932	{ 8	200	20.7	3.4		
	June 12, 1931	{ 8	100	15.0	2.5	6.6	Second cutting, sun-cured; 0.68-inch rain.
Timothy (3)-----	June 24, 1931	{ 8	150	24.0	4.0	6.6	Heads emerging.
	June 24, 1931	{ 8	150	14.2	2.3		
	July 13, 1931	{ 8	300	25.4	4.2		
	June 18, 1931	{ 8	400	13.0	2.2	2.5	Full bloom.
	June 18, 1931	{ 8	400	25.0	4.1		
	July 1, 1932	{ 8	150	9.7	1.6	3.3	Ripe, brown color.
Timothy (3)-----	June 18, 1932	{ 8	300	19.7	3.3	2-3	Early bloom.
	July 1, 1932	{ 8	300	17.4	2.9		
	July 1, 1932	{ 8	450	24.7	4.0	2-3	Late bloom.

¹ The same number indicates that the hays were grown in the same or in adjacent fields.² A unit of vitamin B or G is the weight of the supplement which, when fed to rats under standard conditions will produce an increase in weight of 3 to 4 g per week.³ Estimated, animal could not consume sufficient hay to produce the required increase in weight.⁴ All rats died in 3 weeks.

TABLE 1.—Vitamin B and vitamin G value of hays—Continued

VITAMIN G—Continued

Sample and no.	Date cut	Rats	Level fed daily	Total gain or loss (—) in weight	Weekly gain or loss (—) in weight	Vitamin units per gram (approximate)	Source and description of hay
		No.	Milligrams	Grams	Grams		
Timothy (3), 15150	June 18, 1932	{ 8 8	{ 150 300	{ 7.5 16.0	{ 1.2 2.6	{ 2-3	{ U. S. Timothy Breeding Station, late-maturing variety.
Timothy (3), 12:63	July 1, 1932	{ 8 8	{ 150 300	{ 9.4 18.0	{ 1.5 3.0	{ 3-3	{ Do.
Timothy (3), 12321	June 20, 1933	{ 8 8	{ 100 125	{ 16.0 19.9	{ 2.6 3.3	{ 8.0	{ U. S. Timothy Breeding Station, late-maturing variety, sun-cured.
	June 10, 1932	{ 8	{ 100	{ 24.9	{ 4.1	{ 10.0	{ University farm.
	June 21, 1932	{ 8	{ 100	{ 28.7	{ 4.8	{ 10.0	{ University farm.
Clover (1)-----	June 17, 1932	{ 8 8	{ 200 100	{ 37.8 17.1	{ 6.3 2.9	{ 8 8	{ Cured indoors. 96-hour exposure, no rain.
Alfalfa from alfalfa and timothy mixture (2).	June 8, 1932	{ 8 8	{ 100 150	{ 16.0 21.3	{ 2.7 3.5	{ 6.6	{ Station farm, grown as mixed hay.
Timothy from alfalfa and timothy mixture (2).	do-----	{ 8 8	{ 100 200	{ 15.5 23.3	{ 2.6 3.9	{ 5.0	{ Station farm, grown as mixed hay.
Alfalfa and timothy mixture (50:50) (2).	do-----	{ 8	{ 100	{ 16.4	{ 2.7	{ 6.6	{ Station farm, grown as mixed hay.
Controls-----	-----	16	-----	-2.0	-----	-----	-----

RESULTS

The results of the biological analyses for vitamin B, presented in table 1, show that hays are comparatively low in this factor. Alfalfa (bud stage, May 31) appeared to have a higher vitamin B content than either clover or timothy cut June 10 and 12, respectively, at approximately the same stage of maturity. The data also suggest that the vitamin B content of the plant decreases as the plant matures.

The results of the vitamin G assays (table 1) show that hays may serve as a good source of this vitamin. In general, the vitamin G content was highest in the young plant, and decreased as the plant matured. The relation of the maturity of the plant to its vitamin G content is particularly evident in the case of alfalfa and timothy; clover, however, showed no such relation. Apparently, in the case of the clover the time elapsing between the two cuttings was not long enough to bring out any differences that may have existed between the more and the less mature plants. It is of interest to note that clover and timothy may have as high a vitamin G content as alfalfa when the plants are harvested at corresponding stages of maturity. The comparative vitamin B and G content of alfalfa and timothy cut at the same time is readily seen in the case of the samples grown as mixed hay.

Leaf-hopper injury to alfalfa apparently reduces its vitamin B content. This reduction no doubt is brought about primarily by the loss of leaves. Exposure of alfalfa and clover to the weather (day and night) for 96 hours, more than one-half of which was sunshine, did not seem to affect the vitamin G content of the hay. Rain (0.68 inch), on the contrary, lowered the vitamin G content of alfalfa by 50 percent (see alfalfa (2), second cutting, table 1).

In table 2 an attempt is made to show the relationship between the protein and the vitamin B and G content of hays. It will be observed that, in general, a high protein content is correlated with a high vitamin G content. It is well known that as the plant matures the proportion of leaves to the entire plant decreases and consequently the protein and the vitamin B and G decrease. Accordingly, any procedure or process that would reduce the leaf content of the hay, such as insect injury, excessive handling, or late cutting, would lower the protein and vitamin content and also the quality of the hay.

TABLE 2.—*Protein, crude fiber, and vitamins B and G in hays*

Sample and no. ¹	Date cut	Crude fiber	Protein	Vitamin B units per gram ²	Vitamin G units per gram ²	Description of hay
		<i>Percent</i>	<i>Percent</i>			
Alfalfa (2).....	May 14, 1931	17.42	22.32	2.5	13.0	60 percent leaves.
	June 10, 1931	30.02	13.85	1.8	6.6	40 percent leaves.
	May 31, 1932	25.27	20.14	2.2	10-12	Early bud.
	June 10, 1932	32.41	17.60	2.0	10.0	Early bloom.
Alfalfa (1).....	June 21, 1932	34.76	15.50	1.6	6.6	Late bloom.
	July 28, 1932	32.51	12.91	2.0	6.6	Second cutting, hopper injury.
	Sept. 10, 1932	23.49	19.92	2.0	13.0	Third cutting.
Clover (1).....	June 10, 1932	23.30	15.67	1.6	10.0	Early bloom.
	June 21, 1932	28.73	12.26	1.8	10.0	Late bloom.
Clover (2).....	June 17, 1933		12.13		8	Sun-cured.
	June 12, 1931	23.79	8.50	7-8	6.6	Heads emerging.
Timothy (3).....	June 24, 1931	31.05	7.25	.6	3-4	Full bloom.
	July 13, 1931		5.00	.5	2.5	Brown (ripe) color.
	June 18, 1932	25.65	6.65	1.2	3.3	Early bloom.
	July 1, 1932	26.97	5.21	.6	2-3	Late bloom.
Timothy from alfalfa and timothy mixture	June 8, 1932	23.80	8.66	1.2	5.0	Grown as mixed hay.
Alfalfa from alfalfa and timothy mixture	do	25.95	15.79	1.6	6.6	Do.
Mixed alfalfa and timothy (about 50.50)	do	24.23	10.20	1.4	6.6	Do.

¹ See footnote 1 to table 1.² Approximately.

The correlation of protein and vitamin G content with greenness is only relative and varies with the amount of bleaching due to light, extent of insect injury (leaf hopper), and whether or not the plant is diseased. A similar greenness in alfalfa, clover, and timothy does not necessarily indicate the same vitamin G content. In general, however, the vitamin G values follow in the same order as the protein, leafiness, and greenness of the particular plant.

CHICK EXPERIMENTS

In order to obtain further data on the comparative value of vitamin G in different alfalfa and alfalfa-leaf meals, day-old chicks were used as the experimental animals. These experiments gave a direct comparison of the value of the chick and rat methods of testing for vitamin G.

White Leghorn chicks from the same parent stock were used. They were confined in pens equipped with hardware cloth floors, and fed a ration known to be deficient in vitamin G. Each lot contained 25 chicks. The basal ration was composed of yellow corn, 58 percent; ground wheat, 20; wheat bran, 5; casein (Argentine), 12; steamed bone meal, 3; salt, 1; and cod-liver oil, 1 percent. The meals were incorporated in this ration in the amounts indicated in table 3. The casein and corn were so adjusted that the protein content was approximately the same in all lots. The meals used represented composite

samples of three or more lots, with the exception of the Ohio leaf meals, which involved only one sample. The Ohio leaf-meal fed to lots 10 and 11 (table 3) was prepared from sun-cured hay that had received 0.68 inch of rainfall while in the swath. Liver meal is considered to be a very rich source of vitamin G and for that reason one lot of chicks was fed 3 percent of this as a positive control.

TABLE 3.—*Effect of alfalfa, alfalfa-leaf, and liver meals on growth and leg paralysis of chicks*

Lot no.	Supplement	Protein in meal	Meal consumed per chick in 8 weeks	Rat units of vitamin G per gram of meal	Total rat units of vitamin G consumed per chick in 8 weeks	Average weight per chick in 8 weeks	Leg paralysis	
							Total ¹	Recovered ²
		Percent	Gram			Gram	Percent	Percent
1.....	None					168.8±4.8	67.0	0.0
2.....	5 percent alfalfa meal ³	16.0	38.7	8.0	309.6	278.8±14.0	68.0	8.0
3.....	10 percent alfalfa meal ³	16.0	118.5	8.0	948.0	466.8±13.3	29.0	17.0
4.....	5 percent alfalfa-leaf meal ⁴	20.25	49.9	13.0	648.7	384.6±15.2	48.0	12.0
5.....	10 percent alfalfa-leaf meal ⁴	20.25	130.6	13.0	1,697.8	517.3±11.8	0	—
6.....	5 percent alfalfa meal ⁴	14.25	35.6	8.0	284.8	251.9±11.3	87.0	35.0
7.....	10 percent alfalfa meal ⁴	14.25	119.7	8.0	957.6	454.9±15.0	28.0	16.0
8.....	5 percent alfalfa-leaf meal ⁴	20.56	55.2	13.0	717.6	438.3±14.4	48.0	48.0
9.....	10 percent alfalfa-leaf meal ⁴	20.56	132.4	13.0	1,721.2	528.3±0.9	.0	—
10.....	5 percent alfalfa-leaf meal ⁴ (0.68 inches rain).	20.31	32.8	5.0	164.0	240.6±11.9	83.0	33.0
11.....	10 percent alfalfa-leaf meal ⁴ (0.68 inches rain).	20.31	107.1	5.0	535.5	397.9±13.6	36.0	20.0
12.....	3 percent dried pork liver					602.9±12.3	.0	—

¹ The figures in this column indicate the percentage of chicks that developed leg paralysis during the experiment.

² The figures in this column indicate the percentage of chicks that recovered before the end of the experiment.

³ Meal from hays grown in Ohio.

⁴ Meal from hays grown in Colorado.

It is evident from the data presented in table 3 that the growth of the chicks and the incidence of leg paralysis are directly correlated with the vitamin G content of the meals as determined with rats. Leaf meals having a higher protein and vitamin G content than straight meals caused greater growth and less incidence of leg paralysis than the regular meals of lower protein and vitamin G content. The poor growth and comparatively large percentage of leg paralysis in the case of lots 10 and 11, which received 5 and 10 percent respectively of the leaf meal subjected to rain, suggest that a large part of the vitamin G was lost, and corroborate the results obtained with rats. The data also show that alfalfa meals and leaf meals of approximately the same vitamin G potency produce comparable responses in chicks, whether the meals are produced in Ohio or in Colorado.

A second experiment was conducted partly to check the results of the first trial and partly to compare meals prepared from clover and timothy with those of alfalfa. The same basal ration and procedure used in the first test were employed. The meals were incorporated in the basal ration in amounts shown in table 4. The casein and corn were so adjusted that the total protein content of the rations in all lots

was comparable. The clover and timothy meals were prepared from single samples of hay, while the alfalfa meals represented composite samples of three or more lots. Twenty chicks were started in each lot.

The results presented in table 4 show the ineffectiveness of 10 percent of the straight hay meals in preventing leg paralysis—substantiating the results obtained in the first experiment. Although the results show some variation between different meals, the data in general reveal a close correlation between the response in growth and incidence of leg paralysis and the vitamin G content of the meals as determined on rats. Clover meal gave results comparable to alfalfa meals of the same vitamin G potency but a higher protein content. The results obtained with the timothy meals also compare favorably with those of alfalfa when the vitamin G content of the meals is considered. As in the previous experiment, there was no difference in the results with the Ohio and Colorado meals when compared on the same vitamin G basis.

TABLE 4.—*Effect of alfalfa, clover, timothy, and liver meals on growth and leg paralysis of chicks*

Lot no	Supplement	Protein in meal	Meal consumed per chick in 8 weeks	Rat units of vitamin G per gram of meal	Total rat units of vitamin G consumed per chick in 8 weeks	Average weight per chick in 8 weeks		Leg paralysis	
						Gram		Total ¹	Recovered ²
		Percent	Gram					Percent	Percent
1	None					176.0±5.3		95.0	15.0
2	5 percent clover ³	11.54	38.0	8.0	304.0	243.0±12.3		85.0	35.0
3	10 percent clover ³	11.54	125.7	8.0	1,005.6	453.5±20.2		15.5	0.0
4	5 percent timothy ⁴	7.63	38.9	5.0	194.5	261.8±9.0		65.0	5.0
5	10 percent timothy ⁴	7.63	100.2	5.0	501.0	345.5±11.8		70.0	45.0
6	10 percent timothy ⁵	5.62	92.3	3.0	276.9	314.0±10.6		70.0	25.0
7	5 percent alfalfa meal ⁶	12.25	48.2	8.0	385.6	306.0±11.1		80.0	35.0
8	10 percent alfalfa meal ⁶	12.25	110.8	8.0	886.4	389.8±13.2		40.0	5.0
9	10 percent alfalfa meal ⁷	14.25	101.1	6.6	667.2	368.7±11.9		45.0	35.0
10	5 percent alfalfa meal ⁷	15.56	49.6	8.0	396.8	325.9±11.1		55.0	25.0
11	10 percent alfalfa meal ⁷	15.56	125.7	8.0	1,005.6	430.0±20.2		20.0	10.0
12	3 percent dried pork liver					612.6±9.1		5.0	5.0

The figures in this column indicate the percentage of chicks that developed leg paralysis during the experiment.

² The figures in this column indicate the percentage of chicks that recovered before the end of the experiment.

³ See table 1. Clover cut June 17; 96 hours exposure.

⁴ Timothy cut June 9 (heads emerging). Cured in sun.

⁵ Cut in late-bloom stage.

⁶ Meal from hays grown in Colorado.

⁷ Meal from hays grown in Ohio.

The data in tables 3 and 4 show a close correlation between the total consumption of vitamin G and the weight of the chick and incidence of leg paralysis. The figures in the column showing the total consumption do not include the vitamin G content of the basal ration.

The data obtained by the method used in this investigation appear to show that 10 percent of an alfalfa-leaf meal containing 13 rat units of vitamin G per gram is necessary to induce good growth and prevent the occurrence of leg paralysis.

SUMMARY AND CONCLUSIONS

Alfalfa, clover, and timothy hays cut at different times and cured under different conditions were tested for vitamin B and G with rats as the experimental animals. The results show that these hays contain significantly more vitamin G than vitamin B. The vitamin B and vitamin G content of the hays decreased as the plant matured and, in general, were correlated with the leafiness, greenness, and protein content of the plant.

The exposure of alfalfa to the weather (day and night) for 96 hours—over half of which was sunshine—without rain, did not affect the vitamin G content. Rain (0.68 inch), on the contrary, removed as much as 50 percent of this vitamin.

Timothy and clover cut early may have as high a vitamin G content as alfalfa cut later, and with a much greener color.

The method of testing for vitamin G by growth and incidence of leg paralysis in chicks compared favorably with the rat-assay method.

Ten percent of an alfalfa leaf meal containing 13 rat units of vitamin G per gram was required to induce good growth in chicks and prevent the occurrence of leg paralysis.

LITERATURE CITED

- (1) BETHKE, R. M., and KICK, C. H.
1929. VITAMIN-A CONTENT OF ALFALFA HAY. *Ohio Agr. Expt. Sta. Bull.* 431 (Ann. Rept. 47): 117-118.
- (2) ——— STEENBOCK, H., and NELSON, M. T.
1923. FAT-SOLUBLE VITAMINS. XV. CALCIUM AND PHOSPHORUS RELATIONS TO GROWTH AND COMPOSITION OF BLOOD AND BONE WITH VARYING VITAMIN INTAKE. *Jour. Biol. Chem.* 58: 71-103.
- (3) BOURQUIN, A., and SHERMAN, H. C.
1931. QUANTITATIVE DETERMINATION OF VITAMIN G (B₂). *Jour. Amer. Chem. Soc.* 53: 3501-3505, illus.
- (4) CHASE, E. F., and SHERMAN, H. C.
1931. A QUANTITATIVE STUDY OF THE DETERMINATION OF THE ANTI-NEURITIC VITAMIN B. *Jour. Amer. Chem. Soc.* 53: 3506-3510, illus.
- (5) DOUGLASS, E., TOBISKA, J. W., and VAIL, C. E.
1933. STUDIES ON CHANGES IN VITAMIN CONTENT OF ALFALFA HAY. *Colo. Agr. Expt. Sta. Tech. Bull.* 4, 68 pp., illus.
- (6) HATHAWAY, I. L., DAVIS, H. P., and GRAVES, R. R.
1932. THE VITAMIN A AND THE VITAMIN E CONTENTS OF FIELD-CURED AND ARTIFICIALLY CURED ALFALFA HAY. *Nebr. Agr. Expt. Sta. Research Bull.* 62, 15 pp.
- (7) HAUGE, S. M., and ARTKENHEAD, W.
1931. THE EFFECT OF ARTIFICIAL DRYING UPON THE VITAMIN A CONTENT OF ALFALFA. *Jour. Biol. Chem.* 93: 657-665, illus.
- (8) HUNT, C. H., and KRAUSS, W. E.
1931. THE INFLUENCE OF THE RATION OF THE COW UPON THE VITAMIN B AND VITAMIN G CONTENT OF MILK. *Jour. Biol. Chem.* 92: 631-638, illus.
- (9) RUSSELL, W. C.
1929. THE EFFECT OF THE CURING PROCESS UPON THE VITAMIN A AND D CONTENT OF ALFALFA. *Jour. Biol. Chem.* 85: 289-297.
- (10) STEENBOCK, H., HART, E. B., ELVEHJEM, C. A., KLETZIEN, S. W. F., and RIISING, B. M.
1925. DIETARY FACTORS INFLUENCING CALCIUM ASSIMILATION. VI. THE ANTIRACHITIC PROPERTIES OF HAYS AS RELATED TO CLIMATIC CONDITIONS, WITH SOME OBSERVATIONS ON THE EFFECT OF IRRADIATION WITH ULTRA-VIOLET LIGHT. *Jour. Biol. Chem.* 66: 425-440, illus.

THE INFLUENCE OF CERTAIN DIETARY CONSTITUENTS ON THE RESPONSE OF RATS TO GOSSYPOL INGESTION¹

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INTRODUCTION

Chemical methods for the quantitative determination of gossypol in cottonseed products are based upon the formation of an insoluble compound of gossypol with aniline. In the determination of the gossypol content of raw cottonseeds, the gossypol and oil are extracted from the seeds with diethyl ether. After evaporation of the ether, the residue is taken up with petroleum ether and the gossypol is precipitated with aniline. A positive correlation between the toxicity of raw cottonseeds and their gossypol content has been established by the use of this method (10).²

It has not been possible to establish a similar correlation between the gossypol content and the toxicity of heated cottonseeds or cottonseed meal, principally for two reasons; (1) Only a small portion of the gossypol can be extracted from heated seeds or the meal with the usual fat solvents, and (2) the insoluble portion (so-called D-gossypol or bound gossypol) which may be extracted with hot aniline is of questionable toxicity (4). It has been necessary, therefore, in investigating the gossypol content of these heated cottonseed products to resort to biological methods of assay and from the results so obtained to approximate the amount of physiologically active gossypol which they contain.

In a preliminary study of various methods of biological assay the response of rats to gossypol was found to vary widely depending on the method of administration of the substance (per os and intraperitoneally) and apparently on the nutritive condition of the animals. The most promising method of assay and one of general application was based upon a comparison of the growth rate of rats on diets containing the product under investigation with that of rats on diets of known gossypol content. Later studies revealed that rats were unusually susceptible to gossypol injury while on diets low in vitamin A (6), and in view of the instability of gossypol and its detoxication by soluble iron salts (5) there was reason to believe that the effect of the substance on growth might vary with the nature of the diet in which it was incorporated.

The primary purpose of the present study was to determine what effect alterations in the basal diet might have upon the growth response of rats to gossypol ingestion. Such information was of particular importance in formulating a standard procedure of biological assay. A second purpose, possibly of greater practical importance, was to determine whether certain dietary supplements might act similarly to iron salts in effecting a partial if not complete detoxication of gossypol.

¹ Received for publication Apr. 1, 1935; issued September 1935.

² Reference is made by number (italic) to Literature Cited, p. 266.

METHODS AND EXPERIMENTAL DATA

EXPERIMENTS WITH DIETS OF VARYING PROTEIN CONTENT

The possibility that the deleterious effects of gossypol might be lessened or increased in diets of high protein content was considered first. Accordingly, experimental diets in which the protein was progressively increased from approximately 13 percent to 51 percent were prepared by replacing starch with appropriate amounts of casein. Gossypol was incorporated in the diets by including 15 percent of cottonseeds containing 0.35 percent gossypol. From previous studies it was calculated that this amount of gossypol would not be lethal but would allow the majority of the rats to make slow growth. The percentage composition of the diet of lowest protein content was: Casein 7.4, starch 60.6, Crisco 5.0, salts 3.5, yeast 5.0, cod-liver oil 3.5, and raw cottonseeds 15. The casein was increased to 22, 35, and 51 percent in the diets of higher-protein content.

Control diets of corresponding protein content were prepared in the same way, cottonseeds from which the gossypol had been extracted with ether being used. The oil so removed from the seeds was replaced by an equivalent amount of refined cottonseed oil. The only important difference between the control and the experimental diets so prepared was in their gossypol content. Because of the unstable nature of gossypol, it was deemed necessary to prepare new diets from freshly ground seeds each week.

Young rats weighing between 50 and 60 g which had made uniform growth over a period of a week or 10 days were divided according to litter origin and sex between the gossypol and the control diets. Precautions were taken in selecting the rats to obtain uniform animals and to reduce to a minimum variation in their growth which might be attributed to inheritance or previous nutritional state. For the most part they represented the second, third, and fourth litters of a selected group of healthy females which had been reared and bred for the investigation. They were housed in small individual cages with raised screen bottoms set over circles of paper fitted in the bottom of granite pans. Food was supplied in weighed amounts in feed cups especially constructed to reduce waste. Daily records were kept of the food intake.

As the experiment progressed it was found necessary, because of idiosyncrasies shown by a few animals, to discontinue those rats which at the end of 30 days failed to establish a gain of 30 g. Such animals usually succumbed to the effects of gossypol or lost weight during the next few weeks. Otherwise, the animals were contained on the experimental and control diets for a period of from 60 to 90 days. The results are presented in table 1. The figures for food consumption and gains in weight are average values obtained with an equal number of males and females.

From the results given in table 1, it is apparent that an increase in the protein content of the diet from 13 to 35 percent resulted in an improved rate of growth of the animals on both the gossypol and control diets. A further increase of protein to 51 percent proved less effective for growth of the controls, although on this high level of protein the growth rate of the animals receiving gossypol remained approximately the same as that of those on 35 percent of protein. It is also significant that with a diet containing 35 percent of protein a

large percentage of the animals on the gossypol diets were able to establish the required gain of 30 g during the first 30 days.

It will be observed in table 1 that on each level of protein the animals receiving gossypol consumed less food than did the corresponding controls, although their food intake increased as the amount of protein in the diet was increased to 35 percent. A decrease in food intake is a characteristic effect of gossypol and is attributed not so much to the taste of the compound as to its physiological effect.

To eliminate the effect upon growth resulting from differences in the food intake of the experimental and control rats, the same diets were employed in paired-feeding experiments in which the daily food intake of the animals on the control diet was limited to that of the animals receiving gossypol. The differences in the gains in weight made by the experimental and control animals in each pair in these experiments are, therefore, to be attributed to the effect of gossypol and are unrelated to the plane of nutrition. The results are presented in table 2.

TABLE 1.—Effect of gossypol in the diet on growth of rats, as influenced by the protein content of the diet when the animals were permitted to eat *ad libitum*

Approximate percentage of protein in diet	Gossypol content of diet	Rats started on experiment	Rats continued on experiment	Average food consumed in 60 days	Average gain in weight in 60 days	Difference in gains in weight ¹
	Percent	Number	Number	Grams	Grams	Grams
13.....	0.05	14	10	413	69	64
	(²)	7	7	632	133	
26.....	.05	13	10	485	96	56
	(¹)	8	7	663	182	
35.....	.05	11	9	497	110	60
	(¹)	5	5	712	170	
51.....	.05	13	10	433	108	42
	(²)	7	7	570	150	

¹ In favor of the rats receiving the gossypol-free diet.

² Control, no gossypol.

TABLE 2.—Effect of gossypol in the diet on growth of rats, as influenced by the protein content of the diet when the animals were on paired-feeding tests

Pair no. ¹	Protein content of diet	Gossypol in diet	Duration of experiment	Total food consumed	Total gain in weight	Difference in gain between pair mates ²	Pair no. ¹	Protein content of diet	Gossypol in diet	Duration of experiment	Total food consumed	Total gain in weight	Difference in gain between pair mates ²
	Pct.	Yes... No...	Days	Grams	Grams				Yes... No...	Days	Grams	Grams	
1 M.....	13	Yes... No...	60 60	418 422	77 87	10	9 M.....	35	Yes... No...	90 90	924 926	202 209	7
2 F.....	13	Yes... No...	60 60	535 536	78 104		10 F.....	35	Yes... No...	90 90	796 798	172 187	15
3 M.....	13	Yes... No...	60 60	432 433	62 94	32	11 M.....	35	Yes... No...	90 90	826 827	205 210	5
4 F.....	13	Yes... No...	60 60	376 377	57 69		12 M.....	35	Yes... No...	90 90	873 872	183 200	17
5 M.....	26	Yes... No...	90 90	898 898	155 185	30	13 F.....	51	Yes... No...	60 60	392 393	93 107	14
6 F.....	26	Yes... No...	90 90	870 865	95 108		14 M.....	51	Yes... No...	60 60	586 595	157 173	16
7 M.....	26	Yes... No...	90 90	723 714	154 193	39	15 F.....	51	Yes... No...	60 60	388 387	72 99	27
8 F.....	26	Yes... No...	90 90	623 624	113 137		16 M.....	51	Yes... No...	60 60	503 502	142 148	

¹ Letter following number indicates sex of animals.

² In favor of the rat receiving the gossypol-free diet.

A comparison of the difference in gains in weight between pair mates on the diets of high and low protein content as shown in table 2 affords further evidence of the beneficial effect of a relatively high percentage of protein in diets containing gossypol. Upon a diet containing 35 percent of protein, animals receiving gossypol were able to approximate the gains in weight made by their litter mates on the control diet. At the end of 60 days the average gain in weight made by the male rats on the gossypol diet containing 35 percent of protein was 169 g. As in the preceding experiments, the diets containing 51 percent of protein were less favorable to growth than those containing 35 percent.

These results suggest the possible detoxication of gossypol by protein. Detoxication of gossypol by iron salts is believed to be due to the formation of an iron-gossypol complex which escapes digestion, and it is possible that protein or its degradation products detoxicates gossypol in a similar way. Support for this idea is found in the results of digestion studies carried out *in vitro* by Jones and Waterman (?), who found that the peptic and tryptic digestion of protein was inhibited by gossypol. Schwartz and Alsborg (11) observed that the addition of gossypol to the diet of cats resulted in an increased fecal nitrogen. It has also been suggested that a combination of gossypol with protein accounts for the insoluble condition of gossypol in cottonseed meal (2, 12). None of the evidence so far obtained, however, has been sufficiently conclusive to settle the point. It may be that in the present experiment the increased growth obtained with diets containing 35 percent of protein was due largely to the increased nutritive value of the diets. Nevertheless, considerable importance is attached to these results since they suggest a relationship between protein intake and gossypol injury and explain in part the observations of Clark (3), who found that increasing the casein content of cottonseed-meal diets resulted in an increased rate of growth.³

EXPERIMENTS WITH DIETS OF VARYING FAT AND LACTOSE CONTENT

In view of the favorable results obtained with diets of high protein content, it appeared possible that alterations in the amount of other dietary constituents might likewise prove beneficial.

To determine the effect of altering the fat content of the diet, experimental diets 23, 11, and 22, containing 15 percent of cottonseeds and having a fat content of approximately 7, 12, and 22 percent, respectively, and control diets 11a and 22a, containing 12 and 22 percent of fat, were employed. These diets were fed to groups of rats selected in the manner described in the first experiment. The composition of the diets is given in table 3.

The results of the experiments, which are given in table 4, indicate that a slight improvement in growth is made possible by the inclusion of a large amount of fat in the diet of animals receiving gossypol. The differences in gain in weight between the gossypol and control rats on the high-fat diet and between similar groups of rats on the medium-fat diet were 51 and 56 g, respectively. The differences between the average gains made by the rats on the low- and high-fat diets containing gossypol were not considered sufficiently significant,

³ Since these experiments were completed, Robison (9) has reported the results of studies begun in 1928 to determine the value of cottonseed meal as a protein supplement for growing pigs. As a result of his studies he has set forth the tentative hypothesis that the harmful effect of feeding cottonseed meal to pigs is averted when tankage or a similar protein concentrate is fed, because the gossypol of the meal combines with the protein of the tankage or similar feed to form an insoluble and therefore harmless material.

however, to justify further experimentation with diets of variable fat content. As might be expected, the amount of food required per gram of gain in body weight markedly decreased as the fat content of the diet was increased.

It has been demonstrated that lactose is responsible for a change in intestinal absorption, due possibly to the maintenance of an acid reaction in the intestinal tract (1). The insolubility of gossypol in weak acid suggested the possibility that the addition of lactose to a diet containing gossypol might prove beneficial. Gossypol and control diets containing 20 and 35 percent of lactose were, therefore, employed. Unsatisfactory results were obtained with the higher percentage of lactose due to the development of diarrhea. The results obtained with 20 percent of lactose in gossypol diet 46 and in control diet 46a are given in table 4. From these results it is evident that the addition of lactose not only failed to improve the growth rate of animals receiving gossypol, but produced a decrease in the growth rate of the control animals.

TABLE 3.—Composition of diets used

Components and constituents of diets	Composition ² of diet no.—						
	23	11	22	40 ⁴	46 ³	26	28
Components	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Raw cottonseeds	15.0	15.0	15.0	10.0	10.0	15.0	15.0
Starch	50.7	45.7	35.7	49.0	29.0	44.1	42.7
Casein	22.3	22.3	22.3	24.0	24.0	22.3	22.3
Yeast	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cod-liver oil	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Salt mixture ⁴	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Crisco		5.0	15.0	5.0	5.0	5.0	5.0
Lactose					20.0		
Acid salt mixture ⁵						3.0	
Calcium chloride						1.1	
Calcium carbonate						1.0	2.0
Sodium bicarbonate							1.0
Constituents							
Gossypol	.053	.053	.053	.060	.060	.053	.053
Calcium	.430	.430	.430	.428	.428	1.225	1.230

¹ For each experimental diet made up with raw cottonseeds containing gossypol, a control diet of like composition was made up with gossypol-free seeds. These latter diets are designated in the text and table 4 by the letter *a* following the number of the diet.

² The potential acidity of diets 23, 11, 22, 40, and 46 was equivalent to 170 to 190 cc of N/10 alkali. The potential acidity of diet 26 was equivalent to 447 cc of N/10 alkali. The potential basicity of diet 28 was equivalent to 349 cc of N/10 acid.

³ The gossypol content of the raw seeds used in diets 40 and 46 was 0.60 percent.

⁴ McCollum's salt mixture no. 185 (8).

⁵ The same as McCollum's salt mixture no. 185 except that the calcium lactate (1.30 g) called for in this mixture was replaced by 0.650 g of calcium chloride.

TABLE 4.—Effect of gossypol on growth of rats as influenced by the fat and lactose content and the reaction of the diet when the animals were permitted to eat *ad libitum*

Diet no.	Character of diet	Gossypol in diet	Rats started on experiment	Rats continued on experiment	Average food consumed in 60 days	Average gain in weight in 60 days	Difference in gain in weight ¹
			Number	Number	Grams	Grams	
23	Low fat	Yes	6	5	426	97	56
11	Medium fat	Yes	13	10	485	96	
11a ²	do	No	8	7	603	152	
22	High fat	Yes	12	9	393	104	51
22a ²	do	No	9	7	446	155	
46	20 percent lactose	Yes	8	7	417	81	
46a ²	do	No	6	6	728	125	44
40	No lactose	Yes	21	17	468	85	
40a ²	do	No	6	6	629	160	
26	Acidic	Yes	11	7	415	101	24
26a ²	do	No	11	10	500	125	
28	Basic	Yes	10	9	503	128	
28a ²	do	No	6	6	636	161	33

¹ In favor of the rat receiving the gossypol-free diet.

² Control diet; see footnote 1, table 3.

EXPERIMENTS WITH ACIDIC AND BASIC DIETS

To determine the possible influence of potentially acidic and basic diets on the growth response of rats to gossypol ingestion, calcium chloride and calcium carbonate were added in amounts calculated to yield diets of equal calcium content but of a definitely acid reaction in the one case and alkaline in the other. The amount of calcium chloride used in the first diets tested was too large to permit any of the animals to make consistent gains. The diets rapidly developed a rancid odor and it was found necessary to replace a part of the chloride with an amount of calcium carbonate which would reduce the acidity and yet maintain the calcium content of the diet at the desired level. Rancidity was prevented in these diets by withholding the acid salt mixture from the bulk of the diet and intimately mixing it with each day's supply. The composition of the acidic and basic diets which were finally used and which were found to give the most consistent results is presented in table 3 as diets 26 and 28.

An inspection of the results in table 4 reveals that although the diets made acid by the addition of calcium chloride allowed a majority of the rats on gossypol to make a slight improvement in growth rate they actually proved detrimental to the growth of the control animals. Were it not for the fact that of the 11 animals started on the acidic gossypol diet, 4 were unable to grow during the first 30 days of the experiment, potentially acid diets high in calcium might be considered favorable to the detoxication of gossypol. For example, the control rats on diet 26a made an average gain of only 125 g in 60 days, which was 27 g less than that established by other controls on a similar diet containing less potential acid (diet 11a). However, the gossypol rats on diet 26 made an average gain of 101 g, which was only 24 g less than that made by their controls and 5 g more than that made by rats on a gossypol diet of the same composition exclusive of the acid salt (diet 11).

On the other hand, the use of an alkaline diet prepared by the inclusion of 2 percent of calcium carbonate and 1 percent of sodium bicarbonate (diets 28 and 28a) and having the same calcium content as the acidic diets effected a marked improvement in the growth rate of the rats receiving gossypol and a slight improvement in the growth of their controls. The food intake of gossypol and control animals was increased as a result of this alteration in the diet and it is to be noted that these alkaline diets promoted greater gains and allowed a larger percentage of the animals to survive the experiment than had been possible with any of the previous diets employed. In this dietary series the growth rate of the gossypol animals more closely approached that of the controls than was true in any of the other series with but one exception, that being the acid series, in which the controls made unusually small gains. Of the 10 rats started on the alkaline gossypol diet, 9 finished the experiment with an average gain of 128 g, which was only 33 g less than the average gain made by their controls and 32 g more than that made by rats on a similar diet (diet 11) unsupplemented with the basic salts.

As previously pointed out, precautions had been taken to preserve the integrity of the gossypol molecule in preparing the various diets. Seeds of known gossypol content were used instead of a purified preparation of the isolated substance and fresh diets were frequently pre-

pared. It does not seem likely that destruction of gossypol by its contact with alkali or other reactive constituents in the diet occurred previous to ingestion. Rather, the results lead to the opinion that calcium salts are favorable to the detoxication of gossypol in the animal organism and are particularly effective in the presence of excess base. The possible relationship between calcium metabolism and susceptibility to gossypol injury has been alluded to in an earlier paper (6). Further evidence in support of this opinion has been obtained in experiments carried out *in vitro* in which gossypol, when dissolved in oil and suspended in a buffered alkaline solution, was precipitated upon the addition of a soluble calcium salt. The stability of this calcium complex has not been determined.

SUMMARY AND CONCLUSIONS

The necessity of using biological methods of assay for the determination of physiologically active gossypol in certain cottonseed products prompted a study of dietary factors which influence the response of rats to gossypol ingestion. Accordingly, experimental diets containing a known percentage of gossypol were varied in their content of protein, fat, carbohydrate (lactose), and calcium and in their potential acidity and alkalinity. For each of these alterations made in the gossypol diets, a similar alteration was made in a control gossypol-free diet. The criterion used to judge the effectiveness of a dietary alteration in modifying the deleterious effect of gossypol was that the effect so produced be more apparent in the performance of the rats on gossypol diets than in their controls.

The results of *ad libitum* and paired feeding experiments with diets of variable protein content (13 to 51 percent) indicated that a high protein intake was favorable to the detoxication of gossypol. The effect of high fat diets was questionable. Lactose when fed at a 20-percent level proved to be detrimental to the growth of normal rats; its effect on the gossypol rats was slight. Acidic diets high in calcium (1.2 percent) were likewise detrimental to the growth of normal rats and only slightly influenced the growth of rats which received gossypol and survived the experiment. Alkaline diets high in calcium (1.2 percent) proved to be superior to any of the others in allowing the rats on gossypol to approximate a normal growth rate.

On the basis of these results the conclusion is reached that calcium salts in the presence of excess base are especially favorable to the detoxication of gossypol in the animal organism. Indirect evidence of a chemical reaction between calcium and gossypol is offered in support of this conclusion.

The recommendation is made that in the biological assay of gossypol, a basal diet be employed which is potentially slightly acid and contains only that amount of calcium and protein which is compatible with maintenance and moderate growth. It is tentatively suggested that in feeding cottonseed products to secure rapid growth the ration should contain a reasonable excess of the base-forming elements, particularly calcium, and liberal amounts of protein.

LITERATURE CITED

- (1) **BERGEIM, O.**
1926. **INTESTINAL CHEMISTRY. V. CARBOHYDRATES AND CALCIUM AND PHOSPHORUS ABSORPTION.** Jour. Biol. Chem. 70: 35-45.
- (2) **CLARK, E. P.**
1928. **STUDIES ON GOSSYPOL. II. CONCERNING THE NATURE OF CARRUTH'S D GOSSYPOL.** Jour. Biol. Chem. 76: 229-235.
- (3) ———
1929. **STUDIES ON GOSSYPOL: A PROGRESS REPORT.** Oil and Fat Indus. 6(7): 15-19, illus.
- (4) **GALLUP, W. D.**
1928. **RELATION OF D-GOSSYPOL TO THE TOXICITY OF SOME COTTONSEED PRODUCTS.** Indus. and Engin. Chem. 20: 59-63.
- (5) ———
1928. **THE VALUE OF IRON SALTS IN COUNTERACTING THE TOXIC EFFECTS OF GOSSYPOL.** Jour. Biol. Chem. 77: 437-449.
- (6) ———
1931. **STUDIES ON THE TOXICITY OF GOSSYPOL. I. THE RESPONSE OF RATS TO GOSSYPOL ADMINISTRATION DURING AVITAMINOSIS.** Jour. Biol. Chem. 93: 381-405.
- (7) **JONES, D. B., and WATERMAN, H. C.**
1923. **STUDIES ON THE DIGESTIBILITY OF PROTEINS IN VITRO. IV. ON THE DIGESTIBILITY OF THE COTTONSEED GLOBULIN AND THE EFFECT OF GOSSYPOL UPON THE PEPTIC-TRYPTIC DIGESTION OF PROTEINS.** Jour. Biol. Chem. 56: 501-511, illus.
- (8) **McCOLLUM, E. V., and SIMMONDS, N.**
1918. **A STUDY OF THE DIETARY ESSENTIAL, WATER-SOLUBLE B, IN RELATION TO ITS SOLUBILITY AND STABILITY TOWARDS REAGENTS.** Jour. Biol. Chem. 33: 55-89, illus.
- (9) **ROBISON, W. L.**
1934. **COTTONSEED MEAL FOR PIGS.** Ohio Agr. Expt. Sta. Bull. 534, 44 pp., illus.
- (10) **SCHWARTZ, E. W., and ALSBERG, C. L.**
1924. **RELATION BETWEEN TOXICITY OF COTTONSEED AND ITS GOSSYPOL CONTENT.** Jour. Agr. Research 28: 173-189, illus.
- (11) ——— and **ALSBERG, C. L.**
1924. **PHARMACOLOGY OF GOSSYPOL.** Jour. Agr. Research 28: 191-198, illus.
- (12) **WITHERS, W. A., and CARRUTH, F. E.**
1918. **GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED.** Jour. Agr. Research 12: 83-102, illus.

STUDIES ON THE NICOTINE CONTENT OF CIGARETTE SMOKE¹

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INTRODUCTION

Nicotine has been praised and condemned ever since it was first isolated from tobacco by Posselt and Reimann as cited by Pictet (19a, p. 159)² in 1828. Both praise and condemnation have had little factual basis, however, for the physiological action of nicotine in tobacco smoke is still a controversial subject. Before investigators can establish the effect of inhaling nicotine in smoke, they must know how much of the alkaloid is present. Many conflicting statements are found on this subject.

A review of the literature shows that several factors may cause a fluctuation in the nicotine content of tobacco smoke, among which are the moisture content of the tobacco, the rate of smoking, the quantity left unburned, and the nicotine content of the tobacco itself.

In order to study these factors a satisfactory method for the determination of nicotine in smoke must be employed. The existing methods were therefore reviewed.

METHODS FOR DETERMINING NICOTINE IN TOBACCO AND TOBACCO SMOKE

A method for the determination of nicotine in tobacco smoke must have two particular qualifications. As in the case of tobacco itself, the method must not include the estimation of ammonia, pyridine and its derivatives, or other basic substances as nicotine. It is particularly important that ammonia be separated quantitatively from the nicotine, as shown by the data of Haley, Jensen, and Olson (8)² on cigar smoke. In fact, the ammonia content of smoke equals or exceeds the nicotine content in some cases.³ Schaarschmidt (20) has shown that pyridine is present in much smaller amounts.

A second qualification which the method must have is the ability to measure accurately small amounts of nicotine.

Picric acid reacts with nicotine to give the yellow amorphous dipicrate which gradually changes to the crystalline form. Pfyl and Schmitt (19) have used this reaction as the basis of a method for the determination of nicotine. This method with modifications was used by several German investigators (2, 6, 12, 18, 26), in the analysis of both tobacco and tobacco smoke. Pfyl and Schmitt state that the picric acid method is excellent for the determination of nicotine in tobacco smoke because ammonia, pyridine, and other bases do not interfere. But Koperina (12) states that nitrogen compounds do

¹ Received for publication Dec. 6, 1934; issued September 1935. Submitted in partial fulfillment of the requirements for the degree of doctor of philosophy, published as technical paper No. 654 of the Pennsylvania Agricultural Experiment Station.

² Reference is made by number (italic) to Literature Cited, p. 274.

³ JENSEN, C. O. THE CHEMISTRY OF TOBACCO SMOKE. I. THE STUDY OF THE NICOTINE AND AMMONIA CONTENT OF CIGAR SMOKE. Thesis, M. S., Pa. State Col. 1930.

interfere in this determination. In a comparison of the Pfyl and Schmitt method (19) with Baggesgaard-Rasmussen's silicotungstic acid method (1), Heiduschka and Muth (9) showed that the two methods gave practically identical results.

At the present time Chapin's silicotungstic acid method (7) or modifications of it are widely used for nicotine determinations (3, 23, 4, 11, 16, 22, 23).

Any method in which silicotungstic acid is used as the precipitating agent solves the question of ammonia separation because ammonium silicotungstate is soluble in water (7). Pyridine is more likely to interfere in the precipitation of nicotine (7, 14, 17). However, Mach and Sindlinger (14) state that nicotine can be separated from pyridine in cold 0.5 percent hydrochloric acid solution, if the solution is made so dilute that no pyridine is precipitated. In this case only a very small amount of nicotine escapes precipitation. Baumberger (3) has stated that the nicotine content of smoke can be determined by Chapin's method without pyridine interference. Wenusch (25) used this method on cigar smoke and found that the results obtained were too high. On diluting the solutions to 10 times their original volume before precipitation, the results were the same as those obtained on the polarimeter. It was possible for Wenusch to determine nicotine by the polariscope for he used the smoke from 25 cigars for one determination.

Because of the possible erroneous results that might be obtained if too much pyridine is present, a series of determinations were made to find the concentration at which pyridine does not interfere with the precipitation of nicotine in dilute solutions.

Samples containing nicotine and added amounts of pyridine were treated as outlined by Chapin (7). One hundred cubic centimeters of a solution of nicotine and pyridine was placed in a Kjeldahl flask, made alkaline with sodium hydroxide, and steam-distilled. The solutions obtained were made up to 500 cc, and four 100-cc aliquots were taken. The first aliquot was not diluted but the other three were diluted 1-1, 1-2, and 1-3 with distilled water, acidified with HCl, before precipitation with 5 cc of 12 percent silicotungstic acid. The precipitates were filtered through weighed Gooch crucibles, dried for 3 hours at 125° C., and weighed. The results are given in table 1.

TABLE 1.—*Determination of nicotine as influenced by various concentrations of pyridine*

Solution analyzed	Volume of solution precipitated	Dilution	Concentration of added pyridine	Concentration of nicotine	Weight of precipitate	Nicotine calculated on basis of original sample
	cc		Percent	Percent	Grams	Percent
0.060 g nicotine in 100 cc, no pyridine.....	100	None.	0	0.012	0.1164	0.059
	100	None.	0	0.012	.1168	.059
	200	1-1	0	.006	.1141	.058
	300	1-2	0	.004	.1105	.056
	500	1-4	0	.002	.1070	.054
0.060 g nicotine and 0.20 pyridine in 100 cc.	100	None.	.04	.002	.1649	.083
	200	1-1	.02	.006	.1188	.060
	300	1-2	.013	.004	.1104	.056
	500	1-4	.008	.002	.1075	.054
	100	None.	.12	.012	.5666	.286
0.060 g nicotine and 0.060 g pyridine in 100 cc.....	200	1-1	.06	.006	.3014	.153
	300	1-2	.04	.004	.1649	.083
	500	1-4	.024	.002	.1052	.053

These results show that in solutions containing less than 0.02 percent of pyridine, there is little interference in the estimation of nicotine. However, it is also evident that as the solutions are diluted a small amount of nicotine escapes precipitation. In order, therefore, to be certain that pyridine does not interfere with the determination of nicotine, the solutions must be diluted by trial until there is no sharp drop in the weight of the precipitate.

Solutions obtained from the absorption of cigarette smoke were so treated, and there was no significant difference between the weight of the precipitates from the diluted and the undiluted samples. This agrees with the results of Baumberger (3) and Schaarschmidt and his coworkers (20). Furthermore, after 18 hours the precipitates were crystalline and settled out rapidly after stirring, which is not the case when more than 0.02 percent of pyridine is present.

Since ammonia did not interfere with the results of Chapin's silicotungstic acid method (7) and pyridine did not interfere under the conditions of the writers' tests with rather dilute smoke solutions, this method was used for the present work.

APPARATUS AND PROCEDURE

The machine shown in figure 1 was devised to smoke cigars, cigarettes or pipes with a constant length and strength of puff at the same intervals.

The cigarette *a* is held in a small calcium chloride tube *b* and the smoke is absorbed in the gas-absorption tubes *c*, *d*, *e*, and *f*. The pump *p* creates a partial vacuum in the flask *j*, and this puffs the cigarette whenever the slowly rotating valve *h* is open.

The reducing gear *g* is connected by a short piece of garden hose to the valve *h*. The reducing gear is turned by a synchronous motor *i*. In order to prevent an increase of the vacuum in *j* as measured by the water manometer *m*, air is allowed to bubble through *l* into the bottle *k* which contains oil. The amount of vacuum can be regulated by the height of the oil in *k* and also partly by the valve *n*. The calcium chloride tower *o* is used to prevent water vapor from entering the pump *p*.

Cigarettes were stored for at least 2 weeks in containers of known humidity so that the moisture content could be controlled. Five cigarettes were weighed to the nearest hundredth of a gram and smoked with a "puff" 1.6 seconds long with an interval of 6.1 seconds between puffs. The difference between atmospheric pressure and the pressure in the vacuum bottle was 15.25 inches of water, unless otherwise stated. This allowed 20 cc of air, as measured by a gas meter, to pass through the cigarette with every puff. The smoke was collected in a train of four gas-absorption tubes each containing 12.5 cc of chloroform and 12.5 cc of 0.1 N sulphuric acid.

After the smoke from the five cigarettes had been collected the chloroform-acid solution was placed in a separatory funnel together with the chloroform and water washings from the cigarette holder and the absorption tubes. The liquid was agitated and the lower chloroform layer was removed and discarded, since nicotine sulphate is insoluble in chloroform. The acid solution containing the nicotine sulphate was placed in a Kjeldahl flask and steam-distilled after the addition of 35 cc of sodium hydroxide and a few pieces of porous plate.

The volume of liquid at this point was about 300 to 400 cc. In order to keep the volume of the solution from increasing the flask was heated gently. The distillate was collected in water acidified with 15 cc of dilute HCl (1-4) until 500 cc had distilled over. After the distillation was completed the nicotine was precipitated with 5 cc of 12 percent silicotungstic acid, stirred well, and allowed to stand overnight. It was then filtered through a weighed Gooch crucible, heated in an oven at 125°C. for 3 hours, and weighed. The weight of the precipitate multiplied by the factor 0.1012 gave the weight of nicotine. This was divided by the weight of tobacco smoked to get the weight of nicotine secured from 1 g of tobacco. The data given in this paper are averages of two or more results.

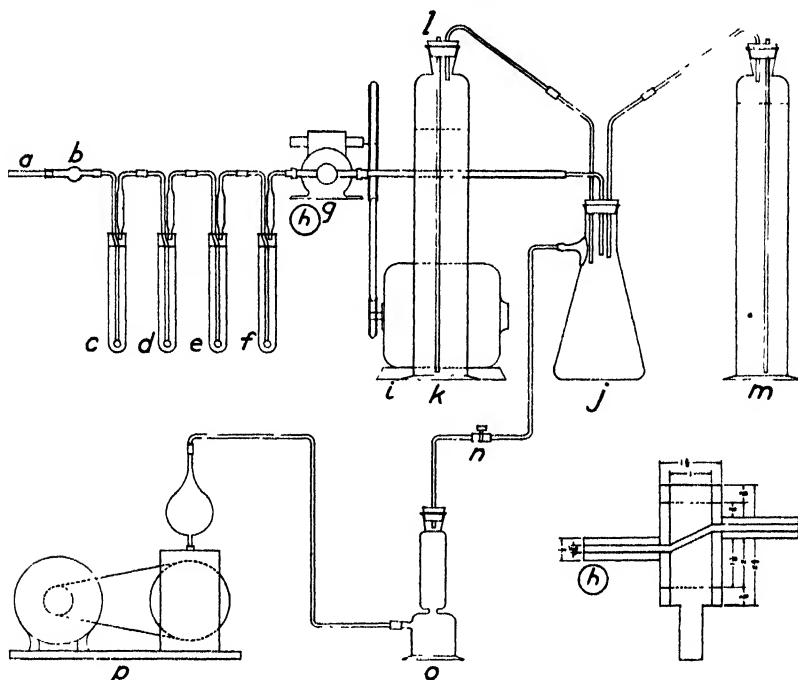


FIGURE 1.—Apparatus used to smoke cigars, cigarettes, or pipes with constant length and strength of puff at same time intervals: *a*, Cigarette; *b*, small calcium chloride tube used as cigarette holder; *c-f*, glass-stoppered gas-absorption tubes, 1 by 8 inches; *g*, reducing gear, 48 to 1, with 8-inch pulley; *h*, brass valve; *i*, synchronous motor, one-sixteenth horsepower, 1,200 revolutions per minute; *j*, 2-liter suction flask; *k*, tall (24-inch) bottle containing motor oil, S. A. E. 30, to a height of 17 inches; *l*, 3.5-mm glass air inlet tube; *m*, tall (24-inch) bottle used as a water manometer; *n*, Hoke needle valve from oxygen tank; *o*, calcium chloride tower; *p*, high-vacuum pump.

The experimental conditions did not of course duplicate actual cigarette smoking conditions.

NICOTINE CONTENT OF CIGARETTE SMOKE AS AFFECTED BY THE MOISTURE CONTENT OF THE TOBACCO

In 1923 Heinz (10) stated that smoke from a moist cigar contained from 50 to 75 percent more nicotine than smoke from a dry cigar. But Schöller's results (21) in 1928 showed practically no difference between the nicotine content of the smoke of dry and moist cigars.

Winterstein and Aronson (29) published results in 1929 which differed from the findings of both of these workers. They state that from a dry cigarette 30 percent more nicotine goes into the mouth of the smoker than from a moist one. The water content of the dry tobacco was 4.8 percent and that of the moist tobacco was 16.5 percent. Waser and Stähli (24) and Molinari (15) also found more nicotine in the smoke from dry cigarettes than from moist ones. Results which differed from any yet reported were given by Kovalenko (13) in 1931, who stated that an increase in the moisture content of cigarettes from 9 to 11 percent increases the nicotine content of the smoke but a further increase in the moisture content decreases the nicotine in the smoke.

EXPERIMENTS

Cigarettes were stored for a period of at least 2 weeks in desiccators having known humidities. The desired relative humidities were obtained from the data of Wilson (27) on humidity control by means of sulphuric-acid solution.

The moisture content of the cigarettes was determined by drying in a vacuum over concentrated sulphuric acid for 2 weeks. The procedure described in the previous section was used to determine the nicotine content of the smoke from these cigarettes. The results are shown in table 2.

TABLE 2.—Nicotine content of cigarette smoke as affected by the moisture content of the tobacco

Moisture content of cigarette (percent)	Nicotine found in smoke per gram of dry tobacco	Nicotine found in the smoke as compared to nicotine in the original tobacco	Moisture content of cigarette (percent)	Nicotine found in smoke per gram of dry tobacco	Nicotine found in the smoke as compared to nicotine in the original tobacco
	<i>Milligrams</i>	<i>Percent</i>		<i>Milligrams</i>	<i>Percent</i>
0.0.....	9.2	42.4	11.10.....	5.7	26.3
3.99.....	7.8	35.9	14.34.....	5.1	23.5
6.43.....	7.3	33.6	24.44.....	4.6	21.2
9.22.....	6.5	30.0			

These results clearly show that an increase in the moisture content of cigarettes decreases the nicotine content of the smoke. Contrary to the findings of Kovalenko (13), the nicotine found in the smoke does not increase with the moisture content from 9 to 11 percent.

NICOTINE CONTENT OF CIGARETTE SMOKE AS AFFECTED BY THE STRENGTH OF PUFF

Bogen (5), Wenusch (26), and Kovalenko (13) all state that the nicotine content of cigarette smoke increases with the rate of smoking.

EXPERIMENTS

Cigarettes stored at four different humidities were smoked under conditions identical with those reported in the previous section, except that the vacuum used was equal to 14 inches of water instead of 15.25 inches. This allowed 16 cc of air to pass through the cigarette at

each puff instead of 20 cc as in the previous series. The results are given in table 3.

TABLE 3.—*Nicotine content of cigarette smoke as affected by the strength of puff, cigarettes of different moisture content being used*¹

Moisture content of cigarette (percent)	Nicotine found in the smoke per gram of dry tobacco		Nicotine found in the smoke as compared to nicotine in the original tobacco	
	16 cc of air puff	20 cc of air puff	16 cc of air puff	20 cc of air puff
	Milligrams	Milligrams	Percent	Percent
0.0.....	8.1	9.2	37.3	42.4
3.99.....	7.0	7.8	32.3	35.9
6.43.....	6.6	7.3	30.4	33.6
31.5.....	2.3	-----	10.6	-----

¹ In these trials 16 cc of air passed through the cigarette at each puff instead of 20 cc as in series reported in table 2.

The results show that an increase in the volume of air going through the cigarette at each puff increases the amount of nicotine in the smoke. This agrees with the findings of others (5, 13, 26).

NICOTINE CONTENT OF CIGARETTE SMOKE AS AFFECTED BY THE LENGTH OF BUTT

Heiduschka and Muth (9) determined the amount of nicotine in the smoke when four-fifths of a cigarette was smoked. Using a 4-second puff at 6-second intervals, they found an average of 0.19 percent of the weight of the cigarettes as nicotine in the smoke. The original nicotine content of the cigarettes used was 1.19 percent.

EXPERIMENTS

Cigarettes of 7-cm length with a nicotine content of 2.17 percent and a moisture content of 11.1 percent were smoked until butts of 1-, 2-, or 3-cm lengths remained. The cigarettes were given a light coating of paraffin near the end and inserted in a warm glass tube which was only slightly larger than the cigarettes. Only the part to be burned remained outside the tube. The paraffin solidified on cooling, forming an air-tight joint. The cigarettes were smoked until the burning zone reached the glass tube. The results are given in table 4.

TABLE 4.—*Nicotine content of cigarette smoke as affected by the length of butt*

Length of butt (length of unsmoked cigarettes, 7 cm) (centimeters)	Fraction of cigarette smoked (<i>F</i>)	Amount of nicotine in smoke per cigarette whose dry weight equals 1 g (<i>A</i>)	Nicotine found in the smoke as compared to nicotine in the original tobacco	Nicotine condensed in butt ($\frac{5.7F-A}{5.7F} \times 100$)
		Milligrams	Percent	Percent
3.....	$\frac{4}{7}$	1.3	6.0	60.1
2.....	$\frac{5}{7}$	2.5	11.5	38.6
1.....	$\frac{6}{7}$	3.8	17.5	22.3
0.....	$\frac{7}{7}$	5.7	26.3	0.0

The percentage of nicotine condensed in the butt was calculated from the difference between the amount of nicotine actually found and the nicotine which theoretically should have been found in the smoke $\left(\frac{5.7 F - A}{5.7 F} \times 100 \right)$.

The condensation of nicotine in the unburned tobacco is a very important factor governing the amount of the alkaloid that the smoker will receive. Under the conditions of this experiment the condensation amounts to 60 percent of the nicotine which ordinarily appears in the smoke, when the length of unburned cigarette is 3 cm or three-sevenths of the original product. With a length of butt of 1 cm, only 22 percent of the nicotine which ordinarily appears in the smoke was held in the unburned portion. The actual amount of nicotine found in the smoke of 1 cigarette burned to a length of 1 cm will be approximately equal to the nicotine found in the smoke from 3 cigarettes whose butts are 3 cm in length. One cigarette smoked to a 2-cm butt will have as much nicotine in its smoke as 2 cigarettes smoked to a length of 3 cm. Although the above exact relationships will hold true only under the conditions of this experiment, it is apparent that the length of butt is one of the most important factors governing the nicotine content of cigarette smoke.

THE NICOTINE CONTENT OF THE "SIDE STREAM"

Bogen (5) states that the side stream ordinarily constitutes the greater part of the smoke as shown by the carbon-dioxide content. Winterstein and Aronson (28) measured the nicotine and reported that 43 to 62 percent of the total nicotine goes into the side stream.

EXPERIMENTS

Cigarettes were placed in the machine and smoked as usual, but instead of allowing the side stream to escape, it was trapped and passed through four gas-absorption tubes. In order to trap the side stream the burning cigarette was placed in a bulb with two small openings at opposite sides. Through one of these openings the cigarette was placed. This opening had a diameter 1 cm greater than that of the cigarette holder, which allowed air to enter the bulb at such a rate that none of the smoke was lost. The air and smoke were pulled through the other opening into the gas-absorption tubes by means of a continuously running water pump.

The results are given in table 5.

TABLE 5.—Nicotine content of the side stream smoke as modified by the moisture content of the cigarette

Moisture content of cigarette (percent)	Main stream (amount of nicotine as compared to the nicotine in tobacco)	Side stream (amount of nicotine as compared to the nicotine in tobacco)	Amount of nicotine in tobacco not found in smoke
	Percent	Per cent	Percent
13.....	26.3	31.8	41.9
0.0.....	42.4	28.6	29.0

Contrary to the results of the study of the relation between the moisture content of the tobacco and the amount of nicotine in the main stream, the side stream shows a decrease of nicotine when the moisture content decreases. This may be partly explained by the fact that dry cigarettes burn more quickly than moist ones. Only 8 puffs are required to burn 1 cm of the dry cigarettes, while 15 puffs are required for those with a moisture content of 11.13 percent. In the more rapidly burning dry cigarettes there is evidently greater distillation and less destruction of the nicotine. This accounts for the increased amounts of the alkaloid in the main stream. But the rate at which the tobacco burns when air is not passing through the dry cigarette may not be increased to the extent that it is when air is drawn through it. Thus the shortened time of burning will cause a smaller amount of nicotine to be found in the side stream. A study of the carbon-dioxide content of the two streams of smoke and the temperature of the burning zone might help to explain the foregoing facts.

SUMMARY

A study of methods of determining nicotine, applicable to cigarette-smoke solutions, has shown (1) that pyridine does not interfere in the precipitation of nicotine by silicotungstic acid in concentrations below 0.02 percent, and (2) that the concentration of pyridine in cigarette-smoke solutions is not high enough to interfere with the precipitation of nicotine by silicotungstic acid.

A machine is described which will smoke cigarettes, cigars, or pipes with puffs of constant volume and unvarying length at constant intervals.

The nicotine content of cigarette smoke varies inversely as the moisture content of the cigarettes.

The amount of nicotine in the smoke is directly proportional to the strength of the puff.

There is a marked condensation of nicotine in the short unburned portion of a cigarette.

Under the conditions of these experiments cigarettes with a moisture content of 11.13 percent contained more nicotine in the side stream than in the main stream; cigarettes with a moisture content of 0 contained less nicotine in the side stream than in the main stream.

LITERATURE CITED

- (1) BAGGESGAARD-RASMUSSEN, H.
1915. DIE BESTIMMUNG VON NICOTIN IN TABAK UND TABAKPRÄPARATEN.
Abstract in Chem. Ztg. 39: 25.
- (2) BARTA, L., and TOOLE, E.
1931. MIKROTIETRIMETRISCHE BESTIMMUNG DES NICOTINS IM TABAKRAUCH.
Ztschr. Angew. Chem. 44: 682-683, illus.
- (3) BAUMBERGER, J. P.
1923. THE NICOTINE CONTENT OF TOBACCO SMOKE. Jour. Pharmacol. and
Expt. Ther. 21: 35-46, illus.
- (4) BENVENIGNI, L.
1931. DU DOSAGE DE LA NICOTINE PAR LA MÉTHODE AU SILICOTUNGSTATE.
Mitt. Lebensmitl. Untersuch. u. Hyg. [Switz.] 22: 217-220.
[Abstract in Chem. Abs. 26: 1390. 1932.]
- (5) BOGEN, E.
1929. THE COMPOSITION OF CIGARETS AND CIGARET SMOKE. Jour. Amer.
Med. Assoc. 93: 1110-1114.

- (6) BOLM, F.
1930. ÜBER RAUCHEN, NICOTINGREZZAHLEN UND DIE NICOTINBESTIMMUNG NACH PFYL UND SCHMITT. *Ztschr. Untersuch. Lebensmtl.* 59: 602-606.
- (7) CHAPIN, R. M.
1911. THE DETERMINATION OF NICOTIN IN NICOTIN SOLUTIONS AND TOBACCO EXTRACTS. U. S. Dept. Agr., Bur. Anim. Indus. Bull. 133, 22 pp.
- (8) HALEY, D. E., JENSEN, C. O., and OLSON, O.
1931. A STUDY OF THE AMMONIA CONTENT OF CIGAR SMOKE. *Plant Physiol.* 6: 183-187, illus.
- (9) HEIDUSCHKA, A., and MUTH, F.
1928. ÜBER NIKOTIN IM TABAK II. *Pharm. Zentralhalle* 69: [305]-307.
- (10) HEINZ, R.
1923. UEBER DIE GIFTIGKEIT DES TABAKRAUCHES, INSBESONDERE DES ZIGARETTENRAUCHES. *Deut. Med. Wehnschr.* 49: 318-319.
- (11) KASANSKY, B.
1931. MASSANALYTISCHE SCHNELLBESTIMMUNG VON NICOTIN MITTELS KIESEL-WOLFRAMSÄURE. *Ztschr. Analyt. Chem.* 83: 107-114.
- (12) KOPERINA, A. W.
1930. UNTERSUCHUNG DER STICKSTOFFHALTIGEN VERBINDUNGEN DES TABAKRAUCHES. *Biochem. Ztschr.* 219: 258-276, illus.
- (13) KOVALENKO, E.
1931. THE NICOTINE CONTENT OF TOBACCO SMOKE. *State Inst. Tobacco Invest. (U. S. S. R.) Bull.* 81: 77-85. [Abstract in *Chem. Abs.* 26: 6066. 1932.]
- (14) MACH, F., and SINDLINGER, F.
1924. VERSUCHE ZU BESTIMMUNG DES PYRIDINS MIT KIESELWOLFRAMSÄURE, INSBESONDERE BEI GEGENWART VON NICOTIN. *Ztschr. Angew. Chem.* 37: 89-92.
- (15) MOLINARI, E.
1932. ÜBER DEN EINFLUSS DER LUFTFEUCHTIGKEIT AUF DEN NIKOTINGEHALT DES TABAKRAUCHES. *Fachl. Mitt. Österr. Tabakregie* 1932(2): 4-7.
- (16) NAGY, V. L.
1932. BESTIMMUNG KLEINER NICOTINMENGEN IM TABAKRAUCH. *Biochem. Ztschr.* 249: [404]-408.
- (17) NORTH, E. O., and BEAL, G. D.
1924. THE PREPARATION, PROPERTIES, AND USES OF SILICODUODECITUNGSTIC ACID. *Jour. Amer. Pharm. Assoc.* 13: 889-898.
- (18) PETRI, W.
1930. ZUR BEURTEILUNG DES NICOTINGEHALTS DER TABAKE. *Ztschr. Untersuch. Lebensmtl.* 60: 123-136.
- (19) PFYL, B., and SCHMITT, O.
1927. ZUR BESTIMMUNG VON NICOTIN IN TABAK UND TABAKRAUCH. *Ztschr. Untersuch. Lebensmtl.* 54: 60-78, illus.
- (19a) PICTET, A.
1904. THE VEGETABLE ALKALOIDS. WITH PARTICULAR REFERENCE TO THEIR CHEMICAL CONSTITUTION. From 2d Fr. ed., rendered into Engl., rev. and enl. . . . by H. C. Biddle. 505 pp. New York and London.
- (20) SCHAARSCHMIDT, A., HOFMEIER, H., and NOWAK, P.
1932. UNTERSUCHUNGEN ÜBER DIE VERWENDBARKEIT VON ADSORPTIONSMITTELEN ZUM ENTGIFTEN VON TABAKRAUCH. *Chem. Ztg.* 56: 911-913, illus.
- (21) SCHÖLLER, R.
1928. NIKOTINGEHALT DES RAUCHES FEUCHTER UND TROCKENER ZIGAREN. *Fachl. Mitt. Österr. Tabakregie* 1927-28 (2): 2-4.
- (22) WAGNER, O.
1932. EINE STANDARDMETHODE ZUR BESTIMMUNG DES NICOTINS. *Chem. Ztg.* 56: 462-463, illus.
- (23) WAKEHAM, G.
1930. RAPID NEPHELOMETRIC METHOD FOR THE APPROXIMATE DETERMINATION OF NICOTINE IN TOBACCO. *Chemist Analyst* 19(1): 8-10.
- (24) WASER, E., and STÄHLI, M.
1932. UNTERSUCHUNGEN AM TABAKRAUCH. I. *Ztschr. Untersuch. Lebensmtl.* 64: 470-485.

-
- (25) WENUSCH, A.
1927. NIKOTINBESTIMMUNGEN IN DEN RAUCHGASEN NORMALER UND ENTNIKOTINISierter ZIGARREN. *Fachl. Mitt. Österr. Tabakregie* 1927-28(1): 7-8.
- (26) ———
1931. BEITRAG ZUR BESTIMMUNG DES NIKOTINS IN RAUCHGASEN. *Fachl. Mitt. Österr. Tabakregie* 1931(1): 1-8.
- (27) WILSON, R. E.
1921. HUMIDITY CONTROL BY MEANS OF SULFURIC ACID SOLUTIONS, WITH CRITICAL COMPILATION OF VAPOR PRESSURE DATA. *Jour. Indus. and Engin. Chem.* 13: 326-331, illus.
- (28) WINTERSTEIN, A., and ARONSON, E.
1928. BEITRÄGE ZUR KENNTNIS DES TABAKGENUSSES. II. MITTEILUNG. ÜBER DEN VERBLEIB DES NICOTINS BEIM TABAKRAUCHEN. *Ztschr. Hyg. u. Infektionskrank.* 108: [530]-553, illus.
- (29) ——— and ARONSON E.
1929. UEBER DAS ZIGARETTENRAUCHEN. *Schweiz. Med. Wehnschr.* 59: 550-552.

THE EFFECT OF AUXINS UPON PHYTOPHTHORA CACTORUM¹

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INTRODUCTION

Ever since Pasteur announced that yeasts were unable to grow in a solution consisting of mineral salts and sugar, investigators have devoted much time not only to the effect of such substances on yeasts and yeastlike fungi, but also to the effect of growth-promoting factors extracted from yeast and tested upon plants and animals. An extensive survey of literature on this subject is given by Miller (6),³ Tanner (12), Sherman and Smith (11), and Buchanan and Fulmer (2). With a few exceptions, filamentous fungi have received but little attention. What few fungi have been tested belong, largely, to the usual genera, such as *Penicillium*, *Aspergillus*, and *Rhizopus*. For instance, Kögl and his coworkers (5), who have isolated three different auxins from human urine and have determined their chemical structure, report the finding of growth-promoting substances in several species of *Rhizopus* as well as in *Escherichia coli*. Nielsen (7) found an auxin in *Rhizopus suinus* and named it rhizopin. According to him this substance accelerates growth of *Aspergillus niger* nine times and greatly increases conidia production. Nielsen and Hartelius (8) made further studies of rhizopin and reported that it contains two distinct auxins, A and B. Auxin A is soluble in ether, is destroyed by oxidation, and promotes growth in oat coleoptile just as Kögl's auxins do. Auxin B, on the other hand, is insoluble in ether, is not destroyed by oxidation, and promotes growth in *A. niger* and not in oat coleoptile. Von Euler and Philipson (4) report that oat coleoptiles contain a growth factor for *R. tritici*, *R. nigricans*, *Aspergillus wentii*, and a species of *Penicillium*. Almoschlechner (1) classifies the auxin found in *Boletus edulis* as "Wuchsstoffe B" which, like auxin B of Nielsen and Philipson, is insoluble in ether. Schopfer (10) found that *Phycomyces blakesleanus* would not grow in a mineral solution and a pure grade of sugar, whereas commercial maltose induced good growth. He tested the effect of vitamins B¹ and B² of Windaus and Kuhn and found that these substances induced growth in the fungus as well as in wheat seedlings.

It is quite probable that a great many fungi are capable of manufacturing their own auxins, while others depend upon other organisms for their growth-promoting substances. For instance, *Mucor genevense*, *Zygorhynchus heterogamus*, *Pythium butleri*, and *Pythium debaryanum* make a good growth in a mineral solution and sugar, whereas *Phycomyces blakesleanus*, *Pythium polymastum*, *Phytophthora cactorum*, and *Coprinus radians* cannot grow unless some complex organic substance is added to the nutrient solution.

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² The writer is indebted to Dr. Virgil G. Lilly for suggestions concerning the chemical phase of the work.

³ Reference is made by number (italic) to Literature Cited, p. 286

EXPERIMENTS

This paper is concerned only with *Phytophthora cactorum* (Leb. and Cohn) Schroeter. The fungus makes no growth whatever when transferred to a solution consisting of 5 g of pure dextrose, 1 g of potassium nitrate, 0.5 g of dihydrogen potassium phosphate, and 0.25 g of magnesium sulphate in 1,000 cc of water. When, however, the primary root of an aseptically germinating corn grain is cut when it is about an inch long and is transferred to this solution, the fungus grows readily (fig. 1) and reproduces both asexually and sexually.

Some may object to the composition of the nutrient solution described above on the ground that it does not contain such essential elements as zinc, manganese, boron, iron, copper, iodine, thallium, etc., which, while needed in very minute quantities, may, nevertheless, be controlling factors in the growth of fungi. In order to overcome this objection, some canned green peas were dried and ashed at a comparatively low temperature; the ash was treated with hydro-

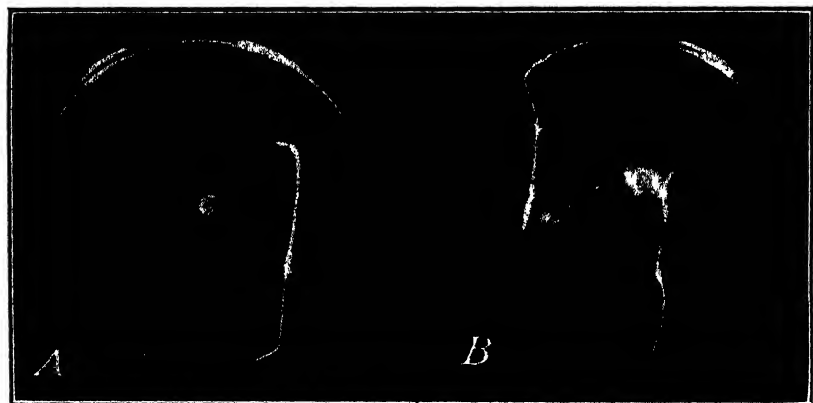


FIGURE 1.—Effect of corn root auxins upon *Phytophthora cactorum*: A, Growth one-sided because there is only one source of auxins; B, A more symmetrical growth because of the presence of two sources of auxins. Note that growth is toward the base of the root where the auxins are given off.

chloric acid, evaporated to dryness on a steam bath, and added to the nutrient solution. It could induce no growth whatever despite the fact that canned peas induce richer and faster growth in *Phytophthora cactorum* than any other substance with which the writer has had experience.

The corn root does not need to remain in the solution for very long; even so short an exposure as 1 minute is sufficient to endow the solution with a certain degree of growth-promoting quality. The longer the root is allowed to remain in the solution, the better the growth will be. But this is not at all proportionate. For instance, the same piece of root was successively transferred through a number of flasks, each containing 15 cc of the nutrient solution. The root was kept in the first flask for only 1 minute, 5 minutes in the second, 10 in the third, 15 in the fourth, 30 in the fifth, 1 hour in the sixth, 2 hours in the seventh, 4 in the eighth, 8 in the ninth, 16 in the tenth, and 24 hours in the eleventh flask. The amount of growth made in the last flask was only three times that made in the first flask. The same was true when a different root was used for each flask.

Went (14) and others have shown that auxins have a definite polarity in the coleoptile of sprouting oat grains, most of the auxins being concentrated at the tip and from there moving toward the base. Popov (9) obtained similar results with corn sprouts. Thimann (13) states that in the roots of *Avena* the concentration of auxins decreases with distance from tip. Cholodny (3) finds that in the case of corn roots transport of growth-promoting substances occurs only in the basal direction. The writer also found that the movement of growth-promoting substances was toward the base, and comparatively smaller amounts were present near the growing tip. This was demonstrated by the following methods. Fifteen cubic centimeters of nutrient medium prepared according to the foregoing formula and solidified by means of 0.75 percent of a pure grade of agar-agar was poured in a series of Petri dishes and sterilized. In the first series of dishes the roots of corn were so placed as to bring the cut base in direct contact with the agar, the remaining parts of the roots being supported above the surface of the agar by means of short pieces of glass tubing. In the second series the position of the root was reversed, and the cut base of the root was sealed with paraffin in order to check any possible diffusion of protoplasmic substances to the surface and from there into the agar. In the third series the position of the root was the same as in the second but the root tip was cut off and the cut end was allowed to rest on the agar. In the fourth series, the position of the root was like that in the first except that the root tip was cut off and sealed with paraffin. All these dishes were then inoculated. The results are shown in figure 2. It can be seen that when the cut base of the root was allowed to come in direct contact with the agar, it induced very good growth; the presence or the absence of the root tip did not seem to have any appreciable effect upon the amount of these substances diffused into the agar. Least growth was made when the sound root tip rested upon the agar; the removal of the root tip caused a somewhat better growth.

Despite the paraffin coating, a certain amount of cell fluid worked its way out from under the paraffin and accumulated on the root in the form of an amber-colored drop. Whenever this drop fell on the agar before it could be absorbed by means of a filter paper, a rich growth ensued. The following technique was used to overcome this: Corn grains were germinated aseptically; when the primary root was about 4 inches long and the shoot had formed, the young plant was removed to a small flask containing the nutrient solution. Only the tip of the root was allowed to come in contact with the solution. In some cases the root tip was cut off; in others it was left intact. The sound roots gave off only a trace of growth-promoting substances and the fungus produced but a slight growth; where the root tip was cut off, a somewhat better growth resulted, but in no case could it compare with the growth induced by a short piece of root aseptically removed from a germinating corn grain and transferred to the nutrient solution. In another series of experiments the corn grains, as soon as they showed signs of germination, were transferred to Petri dishes containing the nutrient medium solidified with agar. The agar was then inoculated; the primary roots were allowed to attain a length of 2 inches. The dishes were then divided into two groups. In the first group the tip of the primary root was

cut off, while in the second group in was left intact. In the case of the former some growth resulted, while in the case of the latter no growth whatever could be seen.

The relative concentration of growth-promoting substances in the roots was tested by another method. When the primary root was about an inch in length, it was cut away from the grain and then cut into two equal parts; the basal portion was transferred to one flask containing the nutrient solution, and the apex to another flask; the

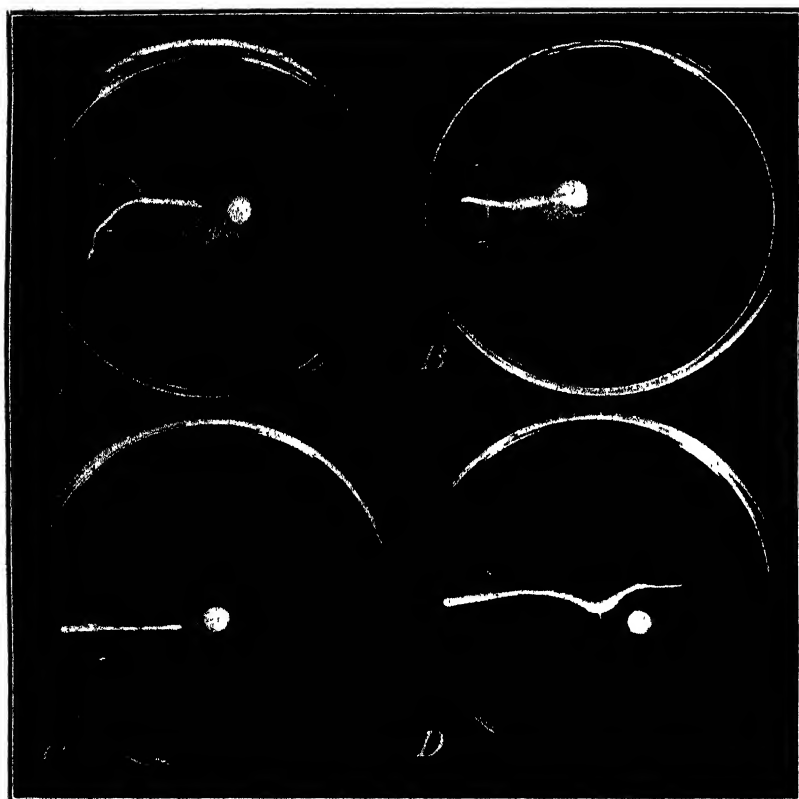


FIGURE 2.—Polarity of auxins: A, The base of the root resting on agar, all other parts being elevated by means of a glass tubing, the root tip intact; B, the base of the root resting on agar, tip cut off; C, the apical portion of the root, with the tip removed, resting on the agar, while the base is elevated; D, the sound apical portion of the root resting on the agar.

flasks were then inoculated. In all cases the basal portion induced a much better growth; even when the root tip of the apical portion was removed and thus two wounded regions were exposed to the solution, no appreciable difference could be seen.

The growth-promoting substances alone, as given off by the roots, cannot support growth. When a piece of root and an inoculum were placed in a dish containing 2 percent agar-agar, no growth resulted. The addition of potassium nitrate, dihydrogen potassium phosphate, and magnesium sulphate, alone or in all possible combinations, induced no growth despite the presence of the root. But the root and dextrose together induced a fairly good growth. However, the

maximum growth resulted only in the presence of the essential salts, glucose, and the root (fig. 3). If the root was left out, no growth resulted.

In all Petri-dish preparations the inoculum was always placed about an inch away from the root. It was observed that the growth of the fungus was always towards the root, so that eventually a fan-shaped colony resulted. No matter how long the fungus was allowed

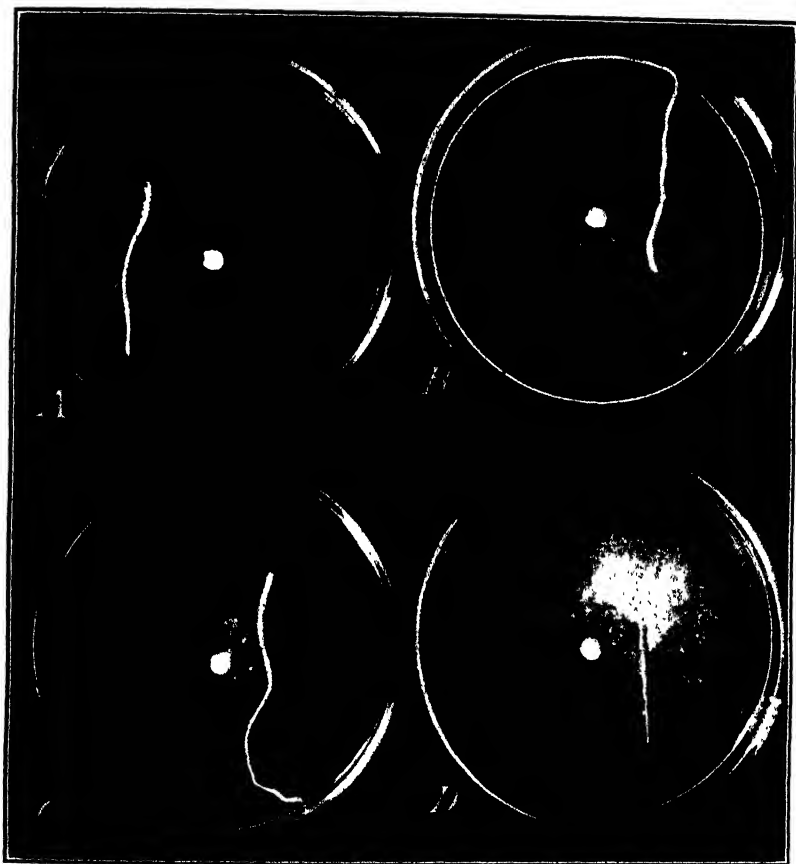


FIGURE 3.—Effect of different nutrients and auxins. A, Plain agar, inoculum, and root, no growth; B, agar, glucose, inoculum, and root, poor growth; C, agar, dihydrogen potassium phosphate, glucose; inoculum, and root, poor growth; D, agar, the essential salts, glucose, inoculum, and root, good growth.

to grow, the colony always remained fan-shaped. This is due to the fact that the mycelium absorbs and retains the growth-promoting substances as rapidly as they are diffused so that one-half of the plate which contains the root will also contain these substances and will, therefore, be able to support growth, while the opposite half, containing no root, will have no such substances and will, therefore, be unable to support growth. Even a casual examination of the colony will show that the richest growth of mycelium is made nearer the root, while the poorest is made in the immediate vicinity of the inoculum; with the exception of those substances which diffuse

through agar and reach the inoculum before any growth is made, all the available supply is absorbed and held by the growing mycelium nearer the source of supply. Even when the root is placed at one edge of the plate and the inoculum at the opposite edge, the picture remains the same, growth becoming more and more sparse as it is followed away from the root. When a strip of agar about 4 mm in width is cut away from the middle of the agar in the plate, leaving the two halves separated by a channel, and when these two halves are connected by an inoculum disk cut from a Petri-dish colony of the fungus by means of a cork borer, growth will be in that half of the agar which contains the root; the other half will show no growth because the young hyphae growing out of the inoculum disk will not allow the growth-promoting substances to diffuse through the inoculum disk into the opposite half of the agar.

It is not at all necessary to have a living root. Roots autoclaved at 15 pounds' pressure for one-half hour and then transferred to the nutrient solution induce a very good growth. When a piece of root is crushed in 15 cc of nutrient solution, excellent growth follows; yet, according to Thimann (13), plant tissues when crushed in water do not yield appreciable amounts of auxins. If the roots are cut and allowed to dry into a brittle, brown tissue and are then transferred to a flask of nutrient solution, again a good growth results. Popov (9) also found that the active principle remained undiminished in dried plants. Thimann, on the other hand, states that root tips of *Avena* do not continue to produce growth substances when cut off and that such substances are easily oxidized and destroyed. The writer wonders if the coleoptile of *Avena* is a very good subject upon which to test auxins, or if a mere curvature and a slight elongation of the shoot is a convincing index for measuring the concentration of auxins. In order to test the effect of oxidation still further, roots were placed in a 1 percent solution of potassium permanganate for 12 hours, then taken out, thoroughly washed, and transferred to flasks containing the nutrient solution. In all cases a good growth followed.

Growth-promoting substances from the corn root can withstand very rough treatments. Some roots were kept for 24 hours in 95-percent alcohol, in full-strength acetone, in chloroform, and in 1-percent solutions of lead nitrate, aluminum nitrate, picric acid, gold chloride, zinc nitrate, and the salts of some other heavy metals known to be strong protein precipitants. After they were washed thoroughly in three changes of sterile distilled water and transferred to the nutrient solution, they induced good growth in the fungus. However, 0.1 percent of bichloride of mercury, 1-percent solutions of copper sulphate and silver nitrate, and full-strength formalin (40 percent) seemed to destroy or inactivate the active principle. It was suspected that the toxic substances were not washed out entirely and that they diffused from the root and inactivated the fungus. Consequently, when the roots treated with bichloride of mercury, copper sulphate, and silver nitrate were first subjected to hydrogen sulphide for 6 hours, boiled in water until all odor of hydrogen sulphide disappeared, and then transferred to the nutrient solution, they induced growth once more. Similarly, when the roots treated with formaldehyde were boiled in distilled water and then transferred to nutrient solution, they gave off enough substances to induce a fairly good growth.

The auxins of the corn root were not destroyed when the roots were boiled in 20-percent sulphuric acid until they began to disintegrate, and were then washed in distilled water and transferred to the nutrient solution. A 12-hour exposure of the roots to N/10 sodium hydroxide solution failed to destroy these substances. A 12-minute exposure to the largest X-ray tube was without any effect (140 pre-voltage, 5 amperes, distance 10 inches).

Adsorption on charcoal, fuller's earth, silica gel, etc., has given good results in the separation of the different fractions of vitamin B and of the so-called "bioses." The writer employed 4-percent norit, an excellent adsorbing agent. A number of flasks, each containing 15 cc of the nutrient solution, were prepared. Each flask contained a piece of root about an inch in length. Ten days later the roots were removed, the solutions combined, and then divided into two equal lots. The first was kept as a check; the second was boiled with 4-percent norit until nearly dry and then made up to the original

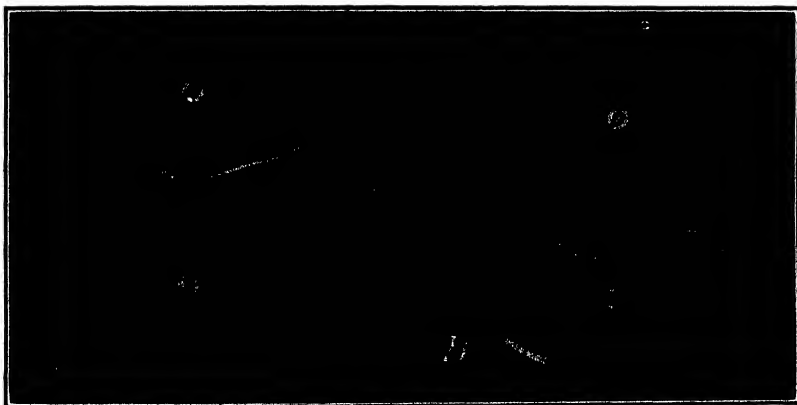


FIGURE 4.—A and B, Effect of adsorption. The black region in the agar consists of nutrient agar to which 4 percent of norit was added. The auxins from the root diffuse in all directions but because they are adsorbed on the norit barrier, inoculum *b* cannot grow, while inoculum *a*, having access to a supply of auxins, grows readily.

volume and filtered. Both the filtrate and the check were autoclaved, poured in a series of sterile glass capsules (preparation dishes), and inoculated. The checks induced a good growth, while the solution treated with norit induced no growth at all, showing that the growth factors were adsorbed on the norit.

The effect of norit was tested also in solid media. The nutrient solution was solidified by means of 2-percent agar-agar. The medium was poured in Petri dishes; after it had solidified, a strip of agar was cut away from the middle of the plate, thus separating the two halves of the nutrient agar by means of a channel. Another portion of the medium, to which 4 percent of norit was added, was poured in this channel. One primary root of corn was placed near this strip of norit-containing agar. The two halves of the untreated agar were then inoculated as indicated in figure 4. It can be seen that the growth factors which diffused through the base of the root were adsorbed on the norit and therefore failed to cross the channel and reach the inoculum *b*; consequently this inoculum failed to grow, while inoculum *a* made a good growth.

The effect of norit-treated auxin solutions upon sexuality of *Phytophthora cactorum* was tested. The writer has already demonstrated that a substance may be able to induce growth, but it may not, necessarily, be able to induce sexuality in this fungus. Proteose peptone is such a substance. When 0.2 percent of proteose peptone is added to the nutrient solution described in a former paragraph, it induces a very good growth but no sexual reproduction. A number of colonies grown in this solution were thoroughly washed in sterile distilled water and transferred to three different solutions, as follows: (1) Nutrient solution containing no auxins; (2) nutrient solution containing auxins; and (3) nutrient solution containing auxins, but heated with 4 percent of norit and filtered. The first solution did not induce any sexual bodies, while the second and the third did. It should be recalled that the second solution induced growth, while the third failed to do so. This indicates that while growth-promoting substances are adsorbed on norit, the sexuality-promoting factors remain unaffected.

NATURE OF THE GROWTH-PROMOTING SUBSTANCES

The term "auxin" is used reservedly in this paper because, while the writer believes that the growth-promoting factors diffused from the corn root are auxins, he has not obtained them in chemically pure form, and the evidence, therefore, is circumstantial in nature. Although ashed peas failed to induce growth, the objection might still be raised that ashing and the subsequent treatment with hydrochloric acid change the various minerals to chlorides and that such minerals may be active only in combination with some organic substances. In cooperation with an organic chemist, Dr. Virgil Lilly, the writer has conducted extensive investigations with growth-promoting substances of the garden pea. Ether and alcoholic extractions containing no minerals whatever have retained all the growth-promoting ability of the original substance. It is, therefore, quite safe to rule out inorganic salts. Similarly, carbohydrates, fats, proteins, proteoses, and amino acids have been left behind without in any way affecting the ability of the refined product to induce as good growth as the original substance.

The substance from the corn root cannot be considered of enzymic nature, for enzymes cannot withstand the violent treatments to which the corn roots were subjected without losing their growth-promoting ability. Nor is it likely that these substances are vitamins. Of the water-soluble types, vitamins B and G are the only ones likely to induce growth. Vitamin B₁ does not resist repeated autoclaving or the action of strong chemicals. The extract from the garden peas which Dr. Lilly and the writer have prepared, purified, and tested, were used by Hazel C. Cameron of this station in some vitamin-G-free diet feeding experiments with rats. The results were all negative, and it is safe to conclude that the growth-promoting factor is not vitamin G.

The growth-promoting substances from the corn roots are not pantothenic acid. The crystalline pantothenic acid obtained through the courtesy of Dr. R. J. Williams failed to induce any growth in *Phytophthora cactorum*. Similarly, one of his "nutrilites" failed in

this respect. Pantothenic acid is a good growth-promoting substance for yeasts; "nutralite 103" promotes growth in some other fungi with which Dr. Williams has experimented. It would, therefore, be an idle generalization to assume that any one auxin may promote growth in all fungi. As previously stated, Kögl and his coworkers have isolated three different auxins from human urine. The one extracted from the garden pea by Dr. Lilly and the writer is different in its chemical properties from auxin A, B, and heteroauxin. There is no reason why there should not be a great many different auxins in nature.

Mitogenetic radiation can at once be waved aside because such radiations, if they actually exist, are associated with living tissues. Corn roots killed by mechanical means, by chemical treatments, and by heat still retain their growth-promoting ability. Furthermore it is difficult to conceive that such rays would be stopped by a narrow strip of agar containing norit (fig. 4).

The definite polarity of the growth factor in the corn root is identical with the behavior of auxins of other investigators. It is not probable that ordinary minerals or organic substances manifest such behavior.

In the light of the foregoing discussion the writer is inclined to favor the theory that the growth-promoting substances present in the corn roots are auxins.

SUMMARY

Canned peas induce an excellent growth and reproduction in *Phytophthora cactorum*. When, however, the peas are ashed, and the ash is added to a mineral solution plus a pure grade of dextrose, the fungus does not grow. But if a piece of root about an inch in length is cut from an aseptically sprouting corn grain and transferred to this solution, the fungus makes a very good growth and reproduces normally.

The root does not need to remain in the solution for very long. Even as short an exposure as 1 minute suffices to endow the solution with growth-promoting properties; however, the growth is richer with the longer exposures.

The growth-promoting substances in the root move toward the base; consequently only very few auxins are given off from the apical portion even when the root tip is cut off. The amount given off from the sound roots is negligible.

The growth-promoting substances as given off by the cut root are unable to induce growth without the essential salts and sugar.

It is not necessary to have living roots. Crushed or autoclaved roots induce excellent growth. Many toxic substances and protein precipitants are without effect; X-rays do not exert any harmful influence.

Growth-promoting factors are adsorbed on norit, so that a norit-treated solution no longer promotes growth. Sexuality-promoting substances, on the other hand, are not adsorbed by norit, so that norit-treated auxin solutions promote sexuality just as well as the untreated ones. This shows that in addition to growth-promoting substances there are sexuality-promoting factors which, apparently possess different chemical and physical properties.

LITERATURE CITED

- (1) ALMOSCHLECHNER, E.
1934. DIE HEFE ALS INDIKATOR FÜR WUCHSTOFFE. *Planta Arch. Wiss. Bot.* 22: [515]-542, illus.
- (2) BUCHANAN, R. E., and FULMER, E. I.
1928-30. *PHYSIOLOGY AND BIO-CHEMISTRY OF BACTERIA*. 3 v., illus. Baltimore.
- (3) CHOLODNY, N.
1934. UEBER DIE BILDUNG UND LEITUNG DES WUCHSHORMONS BEI DEN WURZELN. *Planta Arch. Wiss. Bot.* 21: 517-530, illus. [Abstract in *Chem. Abs.* 28: 6771. 1934.]
- (4) EULER, H. v., and PHILIPSON, T.
1932. WASSERLÖSLICHE WACHSTUMFAKTOREN. *Biochem. Ztschr.* 245: [418]-430. [Abstract in *Chem. Abs.* 26: 3281. 1932.]
- (5) KÖGL, F., HAAGEN-SMIT, A. J., and ERXLEBEN, H.
1932. ÜBER EIN PHYTOHORMON DER ZELLSTRECKUNG. REINDARSTELLUNG DES AUXINS AUS MENSCHLICHEM HARN. 4. MITTEILUNG ÜBER PFLANZLICHE WACHSTUMSSTOFFE. *Hoppe-Seyler's Ztschr. Physiol. Chem.* 214: 241-261, illus.
- (6) MILLER, W. L.
1930. *BIOS*. *Jour. Chem. Ed.* 7: 257-267.
- (7) NIELSEN, N.
1931. THE EFFECT OF RHIZOPIN ON THE PRODUCTION OF MATTER OF *ASPERGILLUS NIGER*. *Compt. Rend. Lab. Carlsberg v.* 19, no. 5, 10 pp.
- (8) ——— and HARTELIUS, V.
1932. THE SEPARATION OF GROWTH-PROMOTING SUBSTANCES. *Compt. Rend. Lab. Carlsberg* 19, no 8, 17 pp.
- (9) POPOFF, M. [POPOV, M.]
1933. UEBER DIE PFLANZLICHEN AUXINE UND IHRE WIRKUNG AUF EINZELLIGE. *Biol. Zentbl.* 53: 661-669. [Abstract in *Chem. Abs.* 28: 1073. 1934.]
- (10) SCHOPFER, W. H.
1934. VERSUCHE ÜBER DIE WIRKUNG VON SEINEN KRISTALLISIERTEN VITAMINEN B AUF *PHYCOMYCES*. *Ber. Beut. Bot. Gessell.* 52: 308-312.
- (11) SHERMAN H. C., and SMITH, S. L.
1931. *THE VITAMINS*. Ed. 2, 575 pp., illus. New York.
- (12) TANNER, F. W.
1925. THE "BIOS" QUESTION. *Chem. Rev.* 1: 397-472.
- (13) THIMANN, K. V.
1934. STUDIES ON THE GROWTH HORMONE OF PLANTS. VI. THE DISTRIBUTION OF THE GROWTH SUBSTANCE IN PLANT TISSUE. *Jour. Gen. Physiol.* 18: 23-34, illus.
- (14) WENT, F. W.
1928. WUCHSTSTOFF UND WACHSTUM. *Rec. Trav. Bot. Néerlandais* 25: 1-114, illus.

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PHOSPHORUS CONTENT AND BUFFER CAPACITY OF PLANT SAP AS RELATED TO THE PHYSIOLOGICAL EFFECT OF PHOSPHORUS FERTILIZERS IN FIBROUS LOW-MOOR PEAT¹

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INTRODUCTION

Ever since the initiation of systematic fertilizer work upon the organic soils of the Everglades, distinctly inimical effects have been observed to follow the use of the more soluble forms of phosphorus under certain conditions on the growth of a considerable number of agricultural plants. In line with this experience, and following the applications of different kinds of phosphatic materials to a series of plots³ on typical saw-grass peat, a planting of shallu (*Andropogon sorghum*) resulted in a distinctly retarded growth on those plots that had received the more soluble forms, such as the superphosphates. Plants on plots that were treated with less soluble materials, such as finely ground rock phosphate, were entirely normal in appearance, however, and much like those to which no phosphorus had been applied.

In an endeavor to ascertain the cause of these disturbances in crop development a study of the sap of the plant was undertaken. This study was extended to embrace a crop of buckwheat (*Fagopyrum esculentum*) grown on the same plots. The growth of this plant was normal on plots treated with phosphorus, but particularly abnormal where phosphorus was omitted and lime added. The same treatment of lime without phosphorus was not detrimental to shallu, however. Corresponding leaf-sap studies of sugarcane (*Saccharum officinarum*), of rape (*Brassica napus*), and of corn (*Zea mays*), grown on similarly treated plots, are also included.

MATERIALS AND METHODS

For the most part leaves only were selected for the sap extractions but in the case of buckwheat and sugarcane, stem saps also were obtained. Samples were always taken between 8 and 9 a. m., and, when leaves were used, they were removed from the same portions of the plants to insure as much uniformity as possible in the sampling procedure. Thus the leaf growing below the last upper well-defined stem internode was selected in the case of the shallu, corn, and sugarcane. The entire buckwheat plant was taken and the leaves were stripped for one sample while the stems were used for another, after discarding the leaf petioles. Samples, usually consisting of about

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² Thanks are due Dr. R. V. Allison, soils specialist in charge, for helpful advice during the course of the work. The writer is also indebted to Dr. A. Daane, agronomist, under whose immediate supervision the crops used in these experiments were grown.

³ Studies on phosphorus sources were begun on these plots in 1929 by Dr. Allison.

100 leaves or stems, were obtained from the central portions of the plots to avoid border effects.

The green plant material was ground in a burr mill and immediately subjected to a pressure of 16,000 pounds per square inch in a steel receptacle. The sap so obtained was centrifuged and portions were used for electrometric titration. When the pH only of the sap was desired a quinhydrone electrode was used. For the establishment of the buffer curves a hydrogen electrode was employed, using 15 ml of the sap and N/5 solutions. Conductivity measurements were made in an Ostwald cell with a Wheatstone bridge and alternating-current galvanometer hook-up. Sap solids were determined with an Abbe refractometer as applied by Gortner and Hoffman (6).⁴ The preparation of sap samples, outlined above, was carried through as rapidly as possible and all reaction measurements were completed within 2 to 3 hours after the sample had been collected. The remainder of the sap was stored in flasks with toluene added as a preservative.

Total phosphorus was determined by evaporating and igniting 5 ml of the sap with magnesium nitrate for the colorimetric molybdenum blue method, amino-naphtol sulphonic acid being used for the reducing agent, as recommended by Fiske and Subbarow (5) and Parker and Fudge (9). Inorganic phosphorus was determined colorimetrically in sap clarified by the method suggested by Emmert (4) for crushed plant tissue. It was found necessary not only to prepare the decolorizing carbon (Norit) by leaching out traces of phosphoric acid with 1 percent H_2SO_4 by volume, but also to wash the precipitate with the diluted acid in order to make sure that there was no occlusion of phosphorus.

The plots, 18 by 100 feet in size, from which the samples used in this work were obtained, were laid out as a duplicate series for 16 different treatments of which 11 are included in this study. In the course of the experiments several different crops were growing at the same time, each planting consisting of a definite number of the 18-foot rows running across each of the 32 plots. The soil is typical of the brown fibrous peat of the Everglades, which, with cultivation, breaks down into a very dark-brown to black, fine-grained, fibrous state. In this locality it is about 7 feet deep and lies directly upon porous limerock with local pockets of marl. The reaction of this soil, undisturbed by burning, ranges between pH 5.6 and 6.0.

The basic fertilizer mixture used in these treatments was a 6-12-12 (N-P-K) combination in which the nitrogen, calculated as N, was derived equally from NaNO_3 and $(\text{NH}_4)_2\text{SO}_4$. The phosphorus sources are recorded below, while the potassium (K_2O) is derived from the sulphate salt. The applications were at the rate of 1,200 pounds per acre, except where colloidal phosphate and rock phosphate were used. In these instances 2 and 4 times the amount of phosphorus (P_2O_5) were added, respectively. Applications were made to these plots on this basis in March 1929, March 1930, April 1931, and September 1932. A general treatment with copper, manganese, and zinc in the form of their sulphates at the rate of 50, 50, and $12\frac{1}{2}$ pounds per acre, respectively, was used upon all plots in accordance with the earlier findings of Allison et al. (1). These were included in the first

⁴ Reference is made by number (italic) to Literature Cited, p. 300.

application only. The treatments made upon the plots included in this study are as follows:

Plot no.	Treatment
1	Check (Cu + Mn + Zn, only, as in all others).
2	K (from sulphate of potash).
3	N + K (from $(\text{NH}_4)_2\text{SO}_4$ and NaNO_3).
4	N + P + K (16-percent superphosphate).
5	N + P + K (45-percent superphosphate).
6	N + 4P + K (raw rock phosphate).
8	N + 2P + K (colloidal phosphate).
9	Check (Cu + Mn + Zn, only, as in all others).
13	N + K + lime.
15	N + P + K + lime (16-percent superphosphate).
16	N + P + K + S (16-percent superphosphate).

Aside from the materials regularly used in the fertilizer mixture, lime was added at the rate of 2,000 pounds per acre; colloidal phosphate at 1,090 pounds; raw rock phosphate, finely ground, at 2,400 pounds; and sulphur at 300 pounds per acre, respectively. The first three of these materials were applied broadcast, while sulphur was incorporated in the fertilizer mixture, drilled in the row with it and worked in. Seven crops had been removed from these plots previous to those under discussion in this paper.

EXPERIMENTAL DATA

RESULTS WITH SHALLU

In the growth of a crop of shallu planted October 8, 1931, a marked physiological disturbance was observed on plots 4 and 5, and especially on plot 16. The first cutting was made on January 14, 1932. This injury consisted of a general stunting of the plants, accompanied by a chlorotic appearance and the inability to tassel and produce seed (fig. 1).

Since the trouble appeared to be associated with the application of soluble phosphates, leaf samples were obtained from plants on plots that represented the various phosphatic treatments. Table 1 gives the results obtained from these samples which were taken on March 19 when the seeds of the plants were in the milk stage. The data show that, whereas there was a moderate increase over the check in sap phosphorus in the leaves from the colloidal phosphate treatment of plot 8, there was a considerably greater increase for the 16-percent superphosphate treatment of plot 4. The amount of phosphorus in the leaf sap of plants from plot 16, to which sulphur was added, was still greater. In contrast, the content of phosphorus in the sap of leaves of shallu plants from the lime plus phosphate plot 15 was but little higher than that from the check plot 2 receiving potash but no phosphate. Characteristics of the saps of plants upon plot 1 must be considered in a special way in this and in subsequent treatments because the lack of potassium in the fertilizer caused the crops to be almost complete failures upon these checks.

Since the increase in total acidity tended to follow that of the total phosphorus (table 1), and since the pH values were but little affected, it would seem that the phosphorus taken up by the plant was exerting a strong buffering action. Where lime was added with the soluble phosphate on plot 15 both growth injury and sap phosphorus were

TABLE 1.—Active and potential acidities and total phosphorus concentrations of leaf saps of shallu from plots upon which different fertilizer treatments were used, Mar. 19, 1932

Plot no.	pH	Acidity ¹	Total P ₂ O ₅	Plot no.	pH	Acidity ¹	Total P ₂ O ₅
		Cc	P. p. m.			Cc	P. p. m.
1-----	5.80	2.55	1,969	8-----	5.87	2.25	2,114
2-----	5.39	1.85	1,713	15-----	5.54	2.40	2,053
4-----	5.71	2.75	2,457	16-----	5.50	5.20	6,471

¹ Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.

reduced. Where the lime was replaced by sulphur in plot 16, plant growth was most seriously retarded. A correlation was particularly evident between growth injury and soluble phosphorus in this instance.

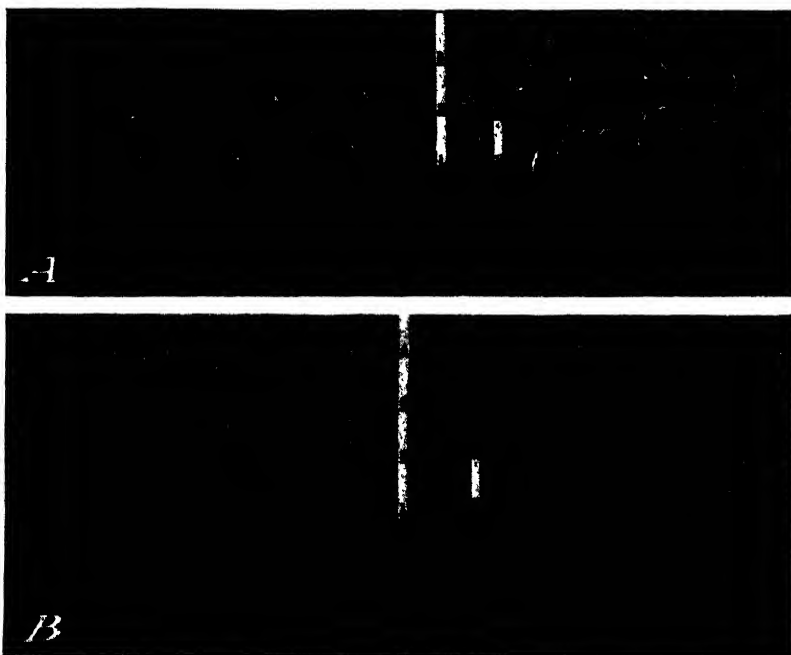
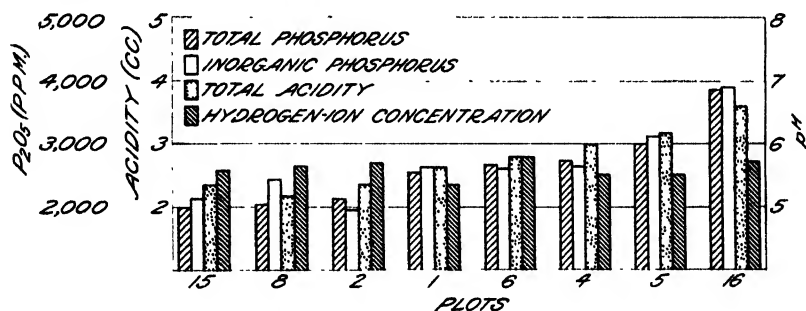


FIGURE 1.—Shallu at the March 19 sampling: A, growth on plot 5 was decidedly retarded because of the inclusion of soluble phosphate in the fertilizer mixture; B, phosphorus was omitted for plot 2, where entirely normal growth was obtained.

The subsequent growth of the second ratoon crop of shallu exhibited appreciably less injury. Leaf samples taken on June 1 at the same stage of growth as those of the previous sampling on March 19 again contained more sap phosphorus when soluble phosphates had been applied as on plots 4 and 16. For plants from plot 4 the amounts were (tables 1 and 2) 2,457 and 2,728 p. p. m. as P₂O₅ and for plot 16 they were 6,471 and 3,824 p. p. m., respectively, for the crops of March 19 and of June 1. Figure 2 shows that almost all of the sap phosphorus was inorganic in nature and that it varied with total acidity. The pH values were practically constant for all of the treatments.

TABLE 2.—*Hydrogen ion concentration, acidity, phosphorus, soluble solids, and conductivity of the expressed leaf saps of corn, shallu, and sugarcane from plots upon which different fertilizer treatments were used, June 1 to 6, 1932*

Crop and plot no - -	pH	Acidity ¹	Total P ₂ O ₅	Inorganic P ₂ O ₅	Soluble solids	Conductivity ²
Shallu		Cc	P p. m.	P p. m.	Percent	Athos
1 - - - - -	5.37	2.60	2,597	2,643	10.53	228
2 - - - - -	5.67	2.35	2,151	1,951	9.43	218
4 - - - - -	5.59	3.00	2,728	2,632	10.13	238
5 - - - - -	5.56	3.20	3,109	3,290	10.83	236
6 - - - - -	5.68	2.70	2,667	2,632	12.03	239
8 - - - - -	5.63	2.20	2,113	2,547	-----	236
15 - - - - -	5.60	2.35	2,057	2,168	9.43	230
16 - - - - -	5.66	3.60	3,821	3,871	9.33	221
Corn						
1 - - - - -	5.63	2.60	985	1,013	10.53	255
2 - - - - -	5.34	3.35	938	880	9.43	263
4 - - - - -	5.41	5.50	1,882	1,796	11.33	258
6 - - - - -	5.73	2.90	2,326	2,300	11.93	240
15 - - - - -	5.59	3.10	1,558	1,536	9.63	215
16 - - - - -	5.51	4.85	2,770	2,667	9.33	234
Sugarcane						
1 - - - - -	5.37	1.75	1,367	1,419	5.53	247
2 - - - - -	5.46	1.40	1,158	1,227	5.43	269
4 - - - - -	5.29	2.48	2,358	2,372	5.45	309
6 - - - - -	5.68	1.35	1,500	1,456	5.33	301
15 - - - - -	5.54	1.50	1,548	1,551	5.36	287
16 - - - - -	5.51	2.30	2,344	2,620	5.23	297

¹ Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.² Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.**FIGURE 2.**—Total phosphorus, inorganic phosphorus, total acidity, and pH values of leaf saps of shallu harvested from variously tested plots June 1. The soil treatments are given in the tabulation on page 289

The third ratoon crop of the shallu was removed September 20. No physiological disturbances were in evidence in the growth of this crop, but the appearance of a leaf-spot disease at the tasseling stage prohibited a definite comparative study of the leaf saps. The phosphorus content of the saps was about the same for all the plots (table 3) and was about as high as that of the harvest of March 19 when growth was so seriously retarded. This point will be discussed later.

RESULTS WITH BUCKWHEAT

A reapplication of fertilizers was made on the plots under study and a crop of buckwheat was planted November 25. Instead of exhibiting injury on plots 4, 5, and 16 where the soluble phosphorus had been added, as was the case with shallu, buckwheat showed a slightly improved growth. Moreover the growth on plot 13 with lime, but without phosphorus, was almost a complete failure.

TABLE 3.—*Leaf-sap analyses of shallu from plots upon which different fertilizer treatments were used, September 20, 1932*

Plot no.—	pH	Acidity ¹	Total P ₂ O ₅	Soluble solids	Amount expressed ²	Conductivity ³	Total dry matter
		Cc	P p. m.	Percent	Percent	Mhos.	Percent
1.....	5.55	2.00	1,990	9.1	37.1	126	26.80
2.....	5.10	2.50	2,048	8.7	29.4	136	34.73
4.....	5.80	2.40	2,335	12.0	34.4	129	30.47
8.....	5.81	1.70	2,326	11.3	27.6	132	31.06
15.....	5.87	1.75	2,128	12.8	31.8	120	32.47
16.....	5.83	3.60	-----	13.6	34.8	136	31.53

¹ Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap² Amount expressed per weight of green material³ Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.

Since the physiological reaction of buckwheat to these treatments was in striking contrast to that of the preceding crop of shallu, similar sap studies were made with this crop. The data for leaf saps, as shown in table 4, indicate that the changes in total phosphorus, total acidity, and inorganic phosphorus tend to parallel one another and show marked increases for the plants from the soluble-phosphate plots. Leaf sap from the plants of the limed plot 13, which received no phosphate treatment, was the lowest in phosphorus and was also very low in total acidity. This would seem to indicate that the poor growth on this plot might be due, in part at least, to a lack of phosphorus. A manganese deficiency may have developed also. In this connection it is to be observed that the saps of the buckwheat leaves from nonphosphate plots (table 4) were much lower in phosphate than the leaf saps of shallu from the same plots. This contrast is not in evidence, however, in saps from the plants of the phosphate-treated plots.

The pH values of the leaf saps of buckwheat (table 4) did not vary appreciably in passing from the nonphosphate to the phosphate plots. Electrometric titrations of these saps were carried sufficiently far to construct the titration curves shown in figure 3, A. The sap of leaves from phosphate-treated plot 5 was buffered considerably more than that of leaves from the nonphosphate plot 2 between the pH range of 5.5 and 11.0.

TABLE 4.—*Leaf- and stem-sap analyses of buckwheat from plots upon which different fertilizer treatments were used, Jan. 11, 1933*

Sap and plot no.	pH	Acidity ¹	Total P ₂ O ₅	Inorganic P ₂ O ₅	Conductivity ²	Total dry matter
		Cc	P. p. m.	P. p. m.	Mhos	Percent
Leaf saps:						
2.....	5.52	1.0	528	-----	100	16.37
3.....	5.54	.5	791	-----	-----	13.27
4.....	5.62	2.1	2,395	1,807	-----	13.97
5.....	5.55	2.0	2,170	1,929	92	14.60
6.....	5.89	1.1	925	984	-----	14.29
13.....	5.85	.6	502	-----	-----	14.62
15.....	5.71	.9	1,374	1,310	-----	15.62
16.....	5.60	1.3	2,182	2,041	-----	14.14
Stem saps:						
2.....	4.80	2.0	545	548	137	15.60
3.....	4.70	1.3	553	534	-----	13.81
4.....	4.75	2.7	3,097	2,930	-----	14.21
5.....	4.80	2.4	3,309	3,604	149	14.20
6.....	4.92	2.0	1,750	-----	-----	-----
13.....	4.75	.9	600	580	-----	14.93
15.....	5.02	2.1	2,544	2,694	-----	16.17
16.....	4.78	2.9	3,275	3,478	-----	15.27

¹ Acidity values are cubic centimeter of N/10 NaOH per 5 cc of sap.² Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.

A somewhat similar variation in buffer capacity is in evidence for the saps of the stems from both plots 2 and 5 (fig. 3, B). The initial hydrogen-ion concentrations of these saps were distinctly higher than those from the leaves (fig. 3, A), however, and they were also more strongly buffered in the lower hydrogen-ion concentration ranges. The buffer capacities of the saps in the higher hydrogen-ion concentrations were about the same for both leaf and stem.

The total phosphorus content of the stem saps was found to be greater than that of the leaves, the minimum and maximum values being, for the stem saps, 545 and 3,309 p. p. m., and for the leaf saps, 528 and 2,395 p. p. m. (table 4). For both leaves and stems the total acidities and inorganic phosphates tend to vary rather closely with the corresponding content of total phosphate. Saps of buckwheat plants from the soluble-phosphate plots were about as high in phosphorus as those of the shallu from the same plots.

BUCKWHEAT VERSUS SHALLU

Following buckwheat the plots were planted to peas, after which shallu was again seeded on

April 28, 1933. Characteristic growth disturbances on the soluble phosphate plots 4, 5, and 16 were again noted, except that they were not as severe as those of the March 19, 1932, harvest, discussed above. Titration curves of leaf saps of plants from plots 2 and 5 (fig. 4, A) show that the sap of plants from the nonphosphate plot 2 was naturally well buffered and that the addition of

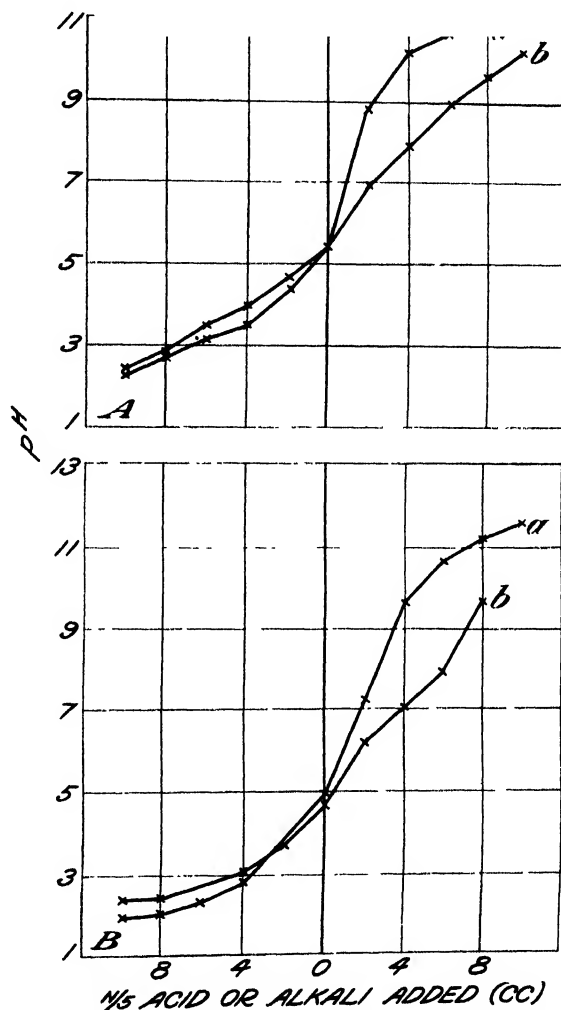


FIGURE 3—Titration curves of 15 ml of leaf sap (A) and stem sap (B) of buckwheat plants, January 11, 1933. Plot 2 (a) received sulphate of potash only and plot 5 (b) received nitrates, sulphate of potash, and 45-percent superphosphate.

soluble phosphorus on plot 5 increased the buffer effect within the range pH 5.0 to above 9.

Figure 4, *B*, shows that the buffer capacity of buckwheat leaf sap is much less than that of shallu. Comparison of figures 3, *A*, and 4, *A*, further indicates that the addition of a soluble phosphate to plot 5 increased the buffer capacity of buckwheat and shallu leaf saps to about the same degree. The total phosphorus in the saps of the shallu leaves was 1,664 and 3,498 p. p. m. (table 5), respectively, for plots 2 and 5. Values from the same plots for the leaf saps of the previous buckwheat crop were 528 and 2,170, respectively (table 4). Further discussion of these points will be taken up in a later section.

TABLE 5.—*Leaf-sap analyses of shallu from plots upon which different fertilizer treatments were used, July 5, 1933*

Plot no	pH	Acidity ¹	Total P ₂ O ₅	Conductivity ²	Total dry matter	Plot no	pH	Acidity ¹	Total P ₂ O ₅	Conductivity ²	Total dry matter
		Cc	P p m.	Mhos.	Percent			Cc	P p m.	Mhos.	Percent
2-----	5.05	1.50	1,664	149	28.88	5-----	4.98	2.70	3,498	135	26.4 ^a
3-----	5.35	1.35	1,941	130	24.84	13-----	5.40	1.30	1,266	160	27.59
4-----	5.59	2.00	2,222	120	22.25	16-----	5.10	2.35	3,489	131	21.86

¹ Acidity values are cubic centimeter of N/10 NaOH per 5 cc of sap.

² Conductivity values equal reciprocal ohms $\times 10^{-1}$ at 28° C.

RESULTS WITH SUGARCANE

Sugarcane of the variety P. O. J. 2725 was planted in April 1931, on plots receiving the treatments outlined above. Table 2 gives the leaf-sap analysis for the sampling made June 1, while table 6 gives analyses for the sampling of August 29 from the same crop. It is to be noted that the sap phosphorus was largely inorganic in nature and that it was considerably higher in leaves from the soluble-phosphate plots 4 and 16. Total acidity values tend to follow those of total phosphorus but do not show a general increase corresponding to the increase in phosphorus in the sap of the more mature leaves of the sampling of August 29. Growth disturbances were distinctly in evidence on plot 16 and to a lesser extent on plot 4.

TABLE 6.—*Leaf- and stem-sap analyses of sugarcane, P. O. J. 2725, from plots upon which different fertilizer treatments were used, Aug. 29, 1932*

Sap and plot no.	pH	Acidity ¹	Total P ₂ O ₅	Soluble solids	Soluble solids per 100 g tissue	Conductivity ²	Total dry matter
Leaves:		Cc	P. p. m.	Percent	Cc	Mhos	Percent
1-----	5.60	1.75	3,670	6.16	55.3	178	28.56
2-----	5.47	1.50	3,100	6.74	55.8	178	27.92
4-----	5.48	2.45	4,651	6.16	58.7	177	25.00
6-----	5.72	2.70	3,947	6.14	55.5	182	28.24
15-----	5.46	1.75	3,658	6.44	51.2	176	27.96
16-----	5.48	2.95	6,608	6.24	53.5	180	25.12
Stems:							
1-----	6.56	.70	320	9.14	83.0	48	15.94
2-----	5.96	.20	465	9.74	82.0	47	16.80
4-----	5.79	.70	1,325	8.74	80.8	53	17.20
6-----	6.09	.40	474	8.74	81.3	62	17.11
15-----	5.95	.20	778	10.04	83.5	48	17.70
16-----	5.54	.90	1,633	9.54	81.5	64	17.75

¹ Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.

² Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.

It is of interest to note that while the stem sap of this variety of sugarcane was found to be much lower in phosphates and in total acidity than that of the leaf (table 6), treatment with soluble phosphates caused a striking increase in these two factors. This marked effect of soil treatment with soluble phosphates upon the concentration of this element in the stem saps of sugarcane was repeatedly observed in other experiments dealing with milling quality.

RESULTS WITH CORN

On February 2, 1932, corn was planted on the entire plot series involving the phosphate sources. Analytical results for leaf samples taken June 4 are recorded in table 2. Corn differed from the other crops studied in that it took up phosphorus about equally well whether this material was applied in the soluble superphosphate form as in plot 4, or as finely ground raw rock phosphate as in plot 6. Lime again depressed the phosphorus content of the sap of the plant (plot 15), while sulphur increased it (plot 16). Results with corn were similar to those with buckwheat in that the absence of a phosphatic fertilizer (plot 2) caused a marked reduction in sap phosphorus. Corn grew well on plot 13 to which lime and potash but no phosphorus was added, under which conditions buckwheat failed almost completely.

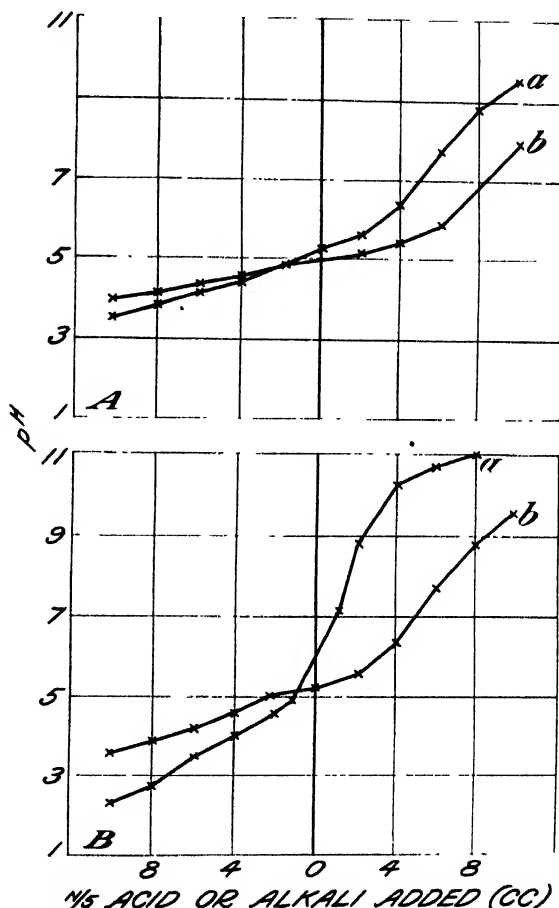


FIGURE 4.—Titration curves of 15 ml. of leaf sap from shallu (A) and from buckwheat (B). Plot 2 (a) received sulphate of potash only and plot 5 (b) received nitrates, sulphate of potash, and 45 percent superphosphate.

RESULTS WITH RAPE

Rape was planted on the entire plot series on December 18, 1932. Marked response to phosphate was shown early in the growth of this plant as recorded in figure 5. Unfortunately buffer-curve data were not obtained but the comparative pH, acidity, and phosphate values for the leaf saps (table 7) indicate that they were in marked contrast with one another, possibly more so than for any of the other crops.

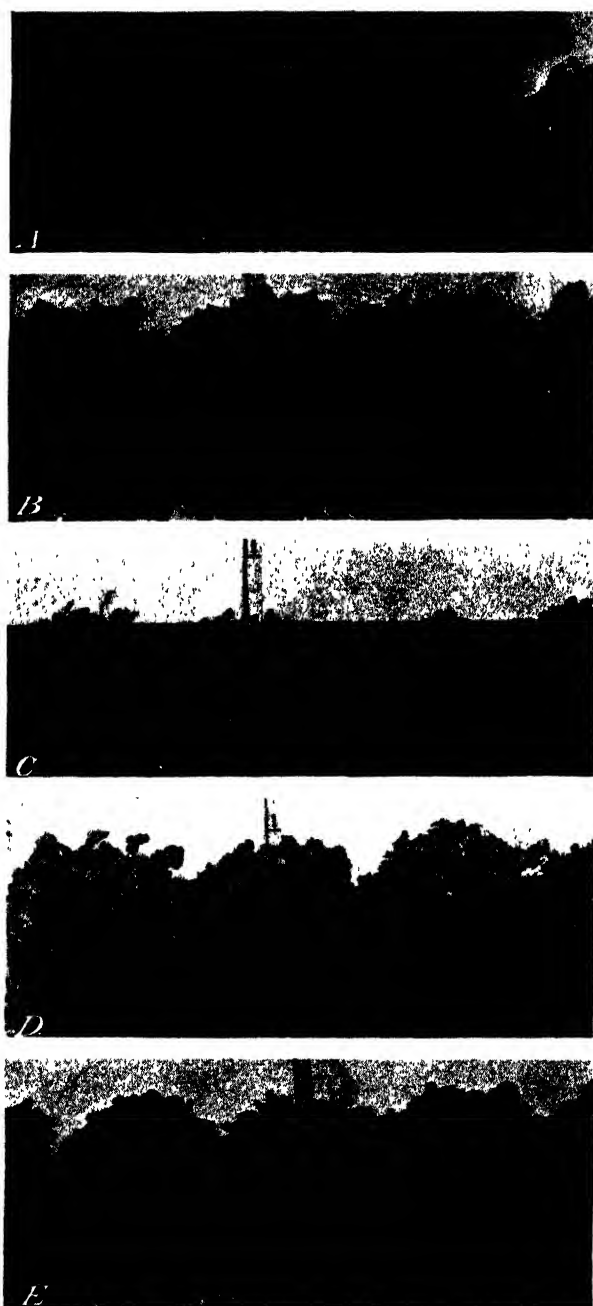


FIGURE 5.—Growth of rape, December 23, on plots all of which received nitrates and potash with the phosphate source varied as shown on page 289: *A*, Plot 3; *B*, plot 4; *C*, plot 13; *D*, plot 15; *E*, plot 16.

TABLE 7.—*Leaf-sap analyses of rape from plots upon which different fertilizer treatments were used, Dec. 23, 1932*

Plot no.	pH	Acidity ¹	Total P ₂ O ₅	Conductivity ²	Total dry matter	Plot no.	pH	Acidity ¹	Total P ₂ O ₅	Conductivity ²	Total dry matter
		Cc	P. p. m	Mhos	Percent			Cc	P p. m.	Mhos	Percent
1.....	6.37	0.65	346	106	10.82	13.....	6.51	0.25	370	138	9.82
3.....	6.40	.50	385	142	11.15	15.....	6.17	.70	764	125	9.01
4.....	6.00	.75	1,961	175	9.99	16.....	6.07	1.00	2,214	135	9.13
6.....	6.39	.50	560	133	9.14						

¹ Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.² Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 25° C.

Table 7 shows that the content of phosphorus was low in the sap of rape plants grown under the nonphosphate plots 3 and 13, and that it was from 5 to 6 times greater in those of the phosphate plots 4 and 16. Increases in plant growth in response to soil treatment with phosphate materials also were striking (fig. 5). The effect of lime was very marked in depressing the phosphate concentration of soluble phosphorus in the sap of the plants (table 7), and caused a near failure of growth on plot 13 which received lime alone without phosphorus treatment of any kind.

SOLUBLE PHOSPHORUS IN PLANTS VERSUS THE PHYSIOLOGICAL EFFECTS OF PHOSPHATE ADDITIONS

Since the growth of shallu and, to a lesser extent, of corn and sugarcane was adversely affected by the application of soluble phosphates whereas buckwheat and rape were distinctly favored by the same treatments, it is of interest to compare certain characteristics of the saps of these plants in an effort to develop some reasonable explanation for the observed differences in response. Perhaps the outstanding sap variation in this connection is that of phosphorus content, and, for purposes of comparison, these values are grouped in table 8. As might be expected, the leaves of shallu, corn, and sugarcane, being less succulent than those of rape and buckwheat, have a higher content of soluble phosphorus. In a similar comparison of plants from plots 2 and 4, untreated and treated with superphosphate, respectively, it is seen that the content of sap phosphorus in the rape and buckwheat was increased nearly 400 percent, whereas in the shallu, corn and sugarcane the increase averaged less than 50 percent (fig. 6).

TABLE 8.—*Sap phosphorus concentration of the leaves and stems of various crops grown on plots fertilized in the same way for each crop*

Plot no.	Shallu		Corn, June 1, leaves	Sugarcane			Rape, Dec. 23, leaves	Buckwheat	
	June 1, leaves	Sept. 20, leaves		June 1, leaves	Aug. 29			Jan. 11	
					Leaves	Stems		Leaves	Stems
	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>
2	2,151	2,048	938	1,158	3,100	465	385	528	545
4	2,728	2,335	1,882	2,358	4,651	1,325	1,961	2,395	3,097
6	2,667		2,326	1,500	3,947	474	580	925	1,750
15	2,067	2,128	1,659	1,588	3,658	778	764	1,374	2,544
16	3,824		2,770	2,344	5,608	1,633	2,214	2,182	3,275

It is also to be observed (fig. 6) that phosphorus concentrations run highest in shallu, the crop most sensitive to an oversupply of phosphorus, while the less sensitive crops, corn and sugarcane, come next in order in this respect. Rape and buckwheat showed the greatest phosphorus responses and had the greatest increases in the phosphorus of the expressed saps. The graphs for all crops are closest together in soluble phosphate for plots 4 and 16, where not only the best growth response was obtained in some but most serious injury occurred in others. From these results it would seem that different plant species may require approximately the same concentration of soluble phosphorus in their saps and that the optimum concentration is also rather near the point of physiological disturbance. Thus, rape and buckwheat might also be adversely affected by this relation in case they assimilated too much phosphorus, a condition that might arise in a more acid soil.

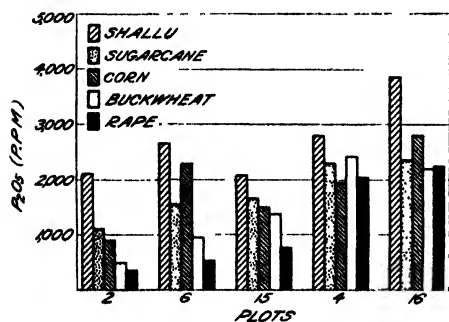


FIGURE 6.—Phosphorus concentration in the leaf saps of shallu June 1; sugarcane June 1; corn June 1; buckwheat January 11; and rape December 23. All plots received potash and all received nitrogen except plot 2. The phosphate source varied as shown in the tabulation on page 289.

SOLUBLE CONCENTRATIONS OF PHOSPHORUS IN PLANTS VERSUS SPECIFIC CONDUCTIVITIES AND TOTAL SOLIDS OF THEIR SAPS

As recorded in tables 2 to 7, analyses of the fresh saps of the crops discussed above also embraced determinations of their specific conductivities. Inspection of these data fails to show any particular relation to phosphate content. This is probably due not only to the contributory influences of other ions, such as Cl, Na, K, Ca, and SO₄, but also to the comparatively low degree of ionization of organic and phosphoric acid radicals.

For a given series of samples the total soluble solids of the saps (tables 2, 3, and 6) varied somewhat but only in an irregular manner. While certain consistent variations occurred as between different kinds of plants and between leaves and stems of the same plants it does not appear that these variations bear any significant relation to the physiological problems under discussion.

DISCUSSION

The assimilation of phosphorus tended to increase very appreciably the total but not the active acidity of the plant saps studied. The buffer capacities of plant saps and the corresponding influence of phosphates have been dealt with from many angles and are reported in a literature much too extensive to be reviewed here. An excellent review of this subject is included in a recent paper by Hurd-Karrer (8). Considering the necessity for ample amounts of phosphorus for plant growth, it is fortunate that phosphate solutions are so well buffered, since only slight changes in pH are possible without causing physiological disturbances. In the present instance, in fact, injury occurred without much change in pH, but rather in the presence of large increases in total acidity and soluble phosphorus. It is signif-

icant to note that almost all of the sap phosphorus was inorganic, as characterized by the presence of the phosphate radical.

As many investigators have pointed out, some plants are able to obtain a sufficient amount of phosphorus for optimum growth from a medium in which other varieties would find insufficient amounts of that element. Under the conditions of the present experiments shallu, corn, and sugarcane are in the first group, since they did not respond to soil treatments with phosphatic materials of any kind, while rape and buckwheat belong to the second group. It is also of interest that the added phosphate caused rape to have a larger growth increase and a considerably greater concentration of phosphorus in the sap than was observed for any of the other crops of this study. Alway, Shaw, and Methley (3) found that increase in the phosphorus content of rape due to fertilization with this element was greater than occurred in any of various crops grown in Minnesota peat soil. Hall (7) has reported that the rutabaga, which belongs to the same genus (*Brassica*) as rape, is a sensitive indicator crop for the need of phosphorus in English soils.

The concentration of phosphorus in the plant saps of the first group, mentioned above, tended to run much higher than in those of the second group when comparisons are made upon plant materials taken from plots to which phosphorus had not been added in any form. Since phosphorus concentrations in the saps of both groups tended to approach each other in the presence of available soil phosphate, it would appear that the optimum concentration for growth is approximately the same for all plants studied. And, since some of the crops, such as shallu, were retarded in growth in the presence of certain of the more soluble phosphate carriers, it would also seem that the point of toxic concentration of this element, when assimilated into the plant, is not far above that of the optimum for normal plant development.

It is perhaps premature to suggest that the presence of high concentrations of soluble inorganic phosphorus in the saps of these plants was entirely responsible for the growth disturbances under discussion. Almost all of the vast amount of literature relative to phosphate fertilizers mention either a positive plant response or none at all. Alway, McMiller, and Rost (2) found, however, that the addition of phosphate alone to a certain type of peat soil resulted not only in no benefit to the growth of any of the crops studied, but actually brought about decreases in the case of corn, flax, and sunflowers. However, phosphate combined with potash increased the yields more than potash alone. It is significant to note that this depressing effect of phosphorus, referred to by Alway, occurred in a peat soil but of a type quite different from that under consideration in this paper. Shive (10) also reports a condition of malnutrition in which monobasic phosphates were, under certain conditions, toxic to soybeans grown in either soil or solution cultures.

The physiological disturbances that were observed in certain of the plants used in these studies appeared to be more severe at some periods than at others. Thus shallu grew more normally at certain seasons when its sap contained as much soluble phosphorus as was found at other times when more serious injury developed. It is thought possible that this inimical effect of soluble forms of phosphorus may be more or less associated with rainfall and soil-moisture conditions. Relations of this type are under observation, and work

is being continued on the phosphorus response of plants growing in organic soils.

SUMMARY

Fresh-sap studies were made of the leaves, and in some cases of the stems, of five different plants growing under field conditions on the characteristic brown fibrous peat of the Everglades. The plots were fertilized in a systematic manner, the most important variable being the carrier of the phosphorus.

The total soluble phosphorus of the saps of these plants was much increased by soil dressings with soluble phosphates. Lime acted to reduce and sulphur to increase the assimilation of phosphorus in all plants studied. Total acidity and the total amount of inorganic phosphorus tended to vary directly with the concentration of soluble phosphorus in the sap. Active acidity of the plant sap, expressed as pH values, changed inappreciably as a result of different phosphatic treatments, whereas the buffer capacities were much greater in the saps of high phosphorus content. Neither the specific conductivities nor the total soluble solids of the saps appeared to have any definite relation either to phosphorus concentration or to physiological injury.

The crops that responded unfavorably, if at all, to phosphorus were those of which the saps were relatively high in phosphorus in the absence of soil treatment with a phosphate carrier. Those that responded favorably, on the other hand, contained sap relatively low in phosphorus.

LITERATURE CITED

- (1) ALLISON, R. V., BRYAN, O. C., and HUNTER, J. H.
1927. THE STIMULATION OF PLANT RESPONSE ON THE RAW PEAT SOILS OF THE FLORIDA EVERGLADES THROUGH THE USE OF COPPER SULPHATE AND OTHER CHEMICALS. (A PRELIMINARY REPORT.) Fla. Agr. Expt. Sta. Bull. 190, pp. [35]-80, illus.
- (2) ALWAY, F. J., McMILLER, P. R., and ROST, C. O.
1921. A SUCCESSFUL COOPERATIVE EXPERIMENT ON A POTASH-HUNGRY PEAT OF DOUBTFUL LIME REQUIREMENT. Jour. Amer. Peat. Soc. 14 (3): 5-18, illus.
- (3) ——— SHAW, W. M., and METHLEY, W. J.
1926. PHOSPHORIC-ACID CONTENT OF CROPS GROWN UPON PEAT SOILS AS AN INDEX OF THE FERTILIZATION RECEIVED OR REQUIRED. Jour. Agr. Research 33: 701-740, illus.
- (4) EMMERT, E. M.
1930. A METHOD FOR THE RAPID DETERMINATION OF PHOSPHATE IN FRESH PLANT TISSUES. Plant Physiol. 5: 413-417.
- (5) FISKE, C. H., and SUBBAROW, Y.
1925. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. Jour. Biol. Chem. 66: 375-400.
- (6) GORTNER, R. A., and HOFFMAN, W. N.
1922. DETERMINATION OF MOISTURE CONTENT OF EXPRESSED PLANT TISSUE FLUIDS. Bot. Gaz. 74: 308-313.
- (7) HALL, A. D.
1905. THE ANALYSIS OF THE SOIL BY MEANS OF THE PLANT. Jour. Agr. Sci. [England] 1: 65-88.
- (8) HURD-KARRER, A. M.
1930. TITRATION CURVES OF ETIOLATED AND OF GREEN WHEAT SEEDLINGS REPRODUCED WITH BUFFER MIXTURES. Plant Physiol. 5: 307-328, illus.
- (9) PARKER, F. W., and FUDGE, J. F.
1927. SOIL PHOSPHORUS STUDIES: I. THE COLORIMETRIC DETERMINATION OF ORGANIC AND INORGANIC PHOSPHORUS IN SOIL EXTRACTS AND THE SOIL SOLUTION. Soil Sci. 24: 109-117.
- (10) SHIVE, J. W.
1918. TOXICITY OF MONOBASIC PHOSPHATES TOWARDS SOYBEANS GROWN IN SOIL- AND SOLUTION-CULTURES. Soil Sci. 5: 87-122, illus.

COMPOSITION OF THE LEAVES AND STALKS OF BARLEY AT SUCCESSIVE STAGES OF GROWTH, WITH SPECIAL REFERENCE TO THE FORMATION OF LIGNIN¹

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INTRODUCTION

In two previous publications (5, 27)³ from the Bureau of Chemistry and Soils, results were presented on the lignin, methoxyl, cellulose, ash, and silica content of the leaves and stalks of lodged and unlodged wheat plants harvested at various stages of growth. The results indicated that the percentage of lignin increased with the age of the plant and that the stalks of the lodged plants in every case contained a higher percentage of lignin than the stalks of the unlodged plants. Because of the general interest in the chemistry of lignin from annual plants, particularly from cereal plants, it seemed advisable to make a more extensive study of the formation of lignin by the plant and to determine, if possible, its interrelationship with other plant components. An opportunity for making such a study was presented when Merritt N. Pope, of the Bureau of Plant Industry, placed at the writers' disposal samples of barley plants harvested daily from the time the plants emerged until maturity. The samples selected for analysis represented for the most part weekly intervals in the development of the plant. At certain stages in the growth of the plant, samples were taken at more frequent intervals.

The present paper deals with a study of the composition of the leaves and stalks of barley at successive stages of growth. Inasmuch as the primary interest in this investigation was the development of lignin in the plant, it seemed best to confine the study to an examination of the several components of the stalk and leaves.

REVIEW OF LITERATURE

Lawes and Gilbert (17) in 1884 analyzed samples of wheat plants taken at different stages of development. They determined the percentages of dry matter, ash, and nitrogen, and found that the percentage of ash decreased steadily as the plants grew older.

Kedzie (14) in 1893 reported that the higher the percentage of crude protein of wheat straw, the lower the percentage of crude fiber and the higher its feed value. As the term "crude fiber" includes a heterogeneous group of chemical substances, his results are difficult to interpret in terms of definite chemical components.

Shaw and Wright (32) studied the chemical composition of sunflower and corn plants at different stages of growth. The percentages of reducing and nonreducing sugars in the sunflower declined some-

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² The writers express their thanks to Merritt N. Pope, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for his kindness in supplying the barley plants used in this investigation.

³ Reference is made by number (italic) to Literature Cited, p. 318.

what irregularly but persistently during the growth of the plant. The percentages of reducing and nonreducing sugars in the corn plant in the early stages of growth were found to vary somewhat, but with a marked upward trend. However, a sudden drop occurred when the kernels were maturing. The percentage of total protein of the sunflower and corn plants decreased as the plants matured.

Ver Hulst, Peterson, and Fred (33) found that the actual weight as well as the percentage of "pentosans" of the corn plant increased as the plant matured. These authors determined the percentage of "pentosans" in the conventional manner, whereby the total furfural obtained when the plant material is distilled with 12 percent hydrochloric acid is calculated as percent pentosans. However, uronic acids and Cross and Bevan cellulose also yield furfural when distilled with acid, so that the values reported by these investigators include several furfural-yielding components calculated as pentosans.

Beckmann, Liesche, and Lehmann (2) determined the lignin content in the leaves and stalks of the rye plant at different stages of growth. Although the method they employed for estimating the percentage of lignin is far from satisfactory, their results indicate that the percentage of lignin and the percentage of methoxyl in the lignin increased as the plant matured.

Dustman and Shriver (7) reported on the chemical composition of *Ambrosia trifida* (giant ragweed) harvested at different stages of growth. The analytical methods were the conventional ones employed in feed-control work. They found that during blooming time, the percentages of crude protein and nitrogen-free extract were the highest. In the later stages the percentage of crude fiber increased. The percentage of pentosan increased as the plant matured.

Phillips, Davidson, and Weihe (27) determined the percentage of lignin, methoxyl, cellulose, methoxyl in the lignin, nitrogen, ash, and silica in the leaves and stalks of lodged and unlodged wheat plants harvested at various stages of growth. Their results indicate that the percentages of methoxyl, lignin, cellulose, and silica in the stalks increased with the age of the plant, whereas the percentages of total nitrogen and ash decreased. The percentages of methoxyl in the lignin from the lodged and unlodged stalks increased at first, but decreased slightly as the plants matured.

Malhotra (18, 19) determined the percentages of moisture, petroleum ether extractives, ash, sugar, starch, hemicelluloses, and total nitrogen in hard winter wheat harvested at successive periods of growth. The percentage of petroleum ether extractives was at maximum during maturity. The percentage of ash was high in the early stages of the development of the plant, then decreased. The percentage of sugar was at a minimum at the beginning; it increased later and finally decreased again. The percentage of the hemicelluloses (determined by hydrolyzing the plant material with 2.5 percent of hydrochloric acid for 4 hours and then determining the reducing sugars formed) increased somewhat during the winter months, then rose rapidly in the spring and finally decreased as the plant matured. Malhotra's results in the hemicellulose content have been criticized by Norman (21) on the ground that the analytical method employed is not specific for this class of substances. The percentage of total nitrogen was lower at first, but it increased during the later stages. It is not

clear, however, whether the figures represent the percentage of nitrogen in the entire plant or only in the straw. Cellulose, pentosans, and lignin were not determined, although these three components constitute the largest percentage of the organic matter of the stalks, particularly at or near maturity.

Norman (21) analyzed barley plants at various stages of growth. The percentages of ash and crude protein showed an initial increase, followed by a steady fall as development proceeded. The percentage of Cross and Bevan cellulose and the percentage of total furfural increased as the plant matured. The percentage of pentose (calculated as arabinose) was irregular, but was lower in the mature plant than in the young plant. The percentage of lignin increased steadily until the last two sampling periods, and then decreased, presumably because of the increased weight of the grain. Norman's figures on the percentage of lignin differ from those recorded in this paper, chiefly owing to the fact that different methods were employed for the quantitative estimation of lignin. These lignin values will be discussed later in this paper, when a comparison is made between the results obtained in this investigation and those recorded by Norman.

There has been considerable speculation concerning the nature of the precursors of lignin. Cross and Bevan (4, pp. 177-181), König and Kump (16, p. 83), and more recently, Fuchs (10, 11) have suggested that cellulose is the parent substance from which lignin is formed. Other investigators, such as Klason (15) and Rassow and Zschenderlein (29) have advanced the hypothesis that lignin is formed by the plant from pentoses or pentosans. The two investigators last mentioned found that plant substances high in lignin were low in pentosans, and vice versa. The possibility that soluble carbohydrates, pentoses, methylpentoses, and hexoses may be used by the plant in the formation of lignin has been suggested by Schrauth (31), Von Euler (9), and Oden (22).

Candlin and Schryver (3) have pointed out that lignified tissues contain lignin and hemicelluloses in relatively large amounts with only traces (if any) of pectins. Nonlignified tissues, on the other hand, contain relatively large amounts of pectin, small amounts of hemicelluloses, and no lignin. They failed, however, to find any direct relationship between pectin and lignin, although by the treatment of pectin with alkali they obtained a product having properties similar to those of the hemicelluloses. More recently, Ehrlich (8) has put forward the hypothesis that pectin is the precursor of lignin. He isolated a fraction from hydropectin which resembled lignin in certain respects; it contained methoxyl to the extent of 11.6 percent, and its percentages of carbon and hydrogen were of the same general order of magnitude as those recorded for lignin. He assumes that enzymatic and chemical reactions take place during the development of the plant to maturity, bringing about the conversion of pectin into lignin.

MATERIAL AND METHODS OF ANALYSIS

The barley plants (*Hordeum distichon palmella*, subvariety Hannchen) used in this investigation were grown under irrigation at Aberdeen, Idaho. The barley seeds were sown on May 9, and the plants emerged on May 19. The age of the plants was determined from

the day they emerged. Samples were taken daily between 8 and 9 a. m. For this investigation, however, most of the analyses were made on samples taken at weekly intervals, although, as previously mentioned, samples were taken more frequently at certain stages in the development of the plants. The plants were taken up by the roots, freed of soil, and air-dried. The roots were cut off, and later, during heading time, the heads were also cut off, and both were discarded. The stalks and leaves were ground fine enough to pass through a 60-mesh sieve, and dried in an oven at 100° C. For the determination of the percentages of alcohol-benzene, cold and hot water, and 1 percent of hydrochloric acid extractives, as well as for the determination of lignin, plant material ground to pass through an 80-mesh sieve was used. The following determinations were made, oven-dried plant material being used for each.

Ash.—This was determined in the usual manner by igniting a weighed sample with a Bunsen burner and weighing the inorganic residue.

Nitrogen.—All nitrogen determinations were made in the usual manner by the Kjeldahl-Gunning-Arnold method (1).

Methoxyl in original plant material.—The percentage methoxyl in the dried unextracted plant material was determined as described by one of the writers in a previous publication (26). The methyl iodide was absorbed in pyridine.

Methoxyl in extracted plant material.—The plant material was successively extracted with a 1 : 2 alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution, and the percentage loss in weight due to these extractions was determined. This operation removed fatty and waxy substances, water-soluble carbohydrates, and proteins, and it also removed any methoxyl groups occurring as methyl esters of organic acids, such as, for example, is found in the pectins. The percentage of methoxyl in the plant material left after extraction was then determined by the method referred to above, and the result was calculated on the basis of the original unextracted material. The percentage of methoxyl thus obtained represents essentially lignin methoxyl, together with some firmly bound methoxyl occurring in some other components of the plant.

Alcohol-benzene extractives.—These were determined by extracting a weighted sample in a Soxhlet extractor for 30 hours with a 1 : 2 alcohol-benzene solution, and ascertaining the loss of weight.

Cold-water extractives.—To a weighed sample of the dry material which had been extracted with alcohol-benzene solution as described above, distilled water was added in the proportion of 150 cc to 1 g of sample and allowed to digest at room temperature with frequent stirring for 48 hours. The loss in weight after extracting and redrying was calculated on the basis of the original unextracted material.

Hot-water extractives.—A weighed sample of the dry residue from the previous extraction was treated with distilled water in the proportion of 150 cc of water to 1 g of sample and boiled under a reflux condenser for 3 hours. The loss in weight was calculated on the basis of the original dry unextracted material.

One-percent hydrochloric acid extractives.—A weighed sample of the plant material which had been successively extracted with alcohol-benzene solution, cold water, and hot water, as described above, was

treated with a 1-percent hydrochloric acid solution in the proportion of 150 cc of acid solution to 1 g of plant material and boiled under a reflux condenser for 3 hours. The loss in weight was calculated on the basis of the original dry unextracted material.

Total extractives.—This represents the sum of the percentages of the alcohol-benzene, cold-water, hot-water, and 1-percent hydrochloric acid extractives.

Uronic acid anhydrides.—These were determined in the dry unextracted material according to the procedure recommended by Dickson, Otterson, and Link (6), as modified slightly by Phillips, Goss, and Browne (28). The uronic acids are present in the pectins and in the hemicelluloses.

Total furfural.—This was determined in the dry unextracted plant material by the method of the Association of Official Agricultural Chemists (1).

Pentosans.—From the percentage of total furfural determined as described above, one-sixth of the percentage of uronic acid anhydrides, determined by the method referred to above, was deducted, and the result was calculated as percentage pentosans. The figure thus obtained represents the sum of the furfural yielded by the pentoses of the polyuronides,⁴ plus the furfural of the Cross and Bevan cellulose, all calculated as percentage of pentosans.

Cross and Bevan cellulose.—This was determined by the method described by one of the writers in a previous publication (26). The percentage of ash in the Cross and Bevan cellulose was determined, and the result was calculated on the basis of ash-free material.

Furfural in Cross and Bevan cellulose.—This was determined by the method of the Association of Official Agricultural Chemists (1). The percentage of furfural was calculated on the basis of ash-free Cross and Bevan cellulose.

Cellulose.—The Cross and Bevan cellulose consists essentially of two components, a "true" cellulose fraction and a furfural-yielding fraction, which in all probability is xylan (20). In order to ascertain the "true" cellulose content of the material, the percentage of furfural in the Cross and Bevan cellulose determined by the method referred to above was calculated as percentage of pentosans, and when this was deducted from the percentage of Cross and Bevan cellulose, the result was the percentage of "true" cellulose in the sample.

Lignin.—The lignin determinations were made on plant material which had been extracted with alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution, according to the procedure described above. The lignin was determined by the fuming hydrochloric acid method described by one of the writers in a previous publication (26). Three samples were weighed, and in the lignin residues from the first two samples the percentages of ash and nitrogen, respectively, were determined; in the lignin residue from the third sample the percentage of methoxyl was determined. Corrections were made for the nitrogen and ash in the lignin.

Methoxyl in ash-free lignin.—The percentage of methoxyl in the lignin residue was determined by the method referred to above, and the result was calculated on the basis of ash-free lignin.

⁴ The term "polyuronides" was introduced into chemical literature by Candlin and Schryver (3). It refers to a group of substances, widely distributed in the plant kingdom, which are formed by the conjugation of certain sugar acids (glucuronic and galacturonic acids) with sugars. The hemicelluloses and the pectins are, therefore, polyuronides.

TABLE 1.—Percentage composition of barley plants at different stages of growth

[Results calculated on the basis of oven-dried material. Figures in parentheses give percentages on the basis of ash-free material]

Age of plants (days)	Ash in original plant material	Nitrogen in original plant material	Methoxy in original plant material	Methoxy in extracted plant material	Alcohol-benzene extractions	Cold water extractions	Hot water extractions	1 percent hydrochloric acid extractions	Total extractives	Uronic acids (as anhydrides)	Total furfural	Pentosans	Cross and Bevan cellulose	Furfural in cross and Bevan cellulose	Cellulose	Lignin	Nitrogen in crude lignin	Methoxy in ash-free lignin	Ash in lignin	Methoxy in "pure" lignin
7 ⁴	13.68	5.87 (6.79)	0.54 (.62)	0.14 (.16)	24.83 (28.76)	17.82 (20.64)	5.74 (6.65)	29.58 (34.26)	77.97 (90.31)	6.16 (7.13)	6.31 (7.31)	9.04 (10.47)				1.48 (1.71)	6.95 (8.87)		21.61	-----
14 ⁵	13.61	5.87 (6.79)	0.38 (.47)	0.14 (.16)	30.48 (35.28)	17.28 (20.00)	4.69 (5.43)	27.23 (31.58)	79.73 (92.29)	5.86 (6.80)	5.86 (6.78)	8.34 (9.65)				1.71 (1.98)	6.76 (8.62)	2.03	21.61	4.42
21	16.48	6.08 (7.28)	0.69 (.70)	0.15 (.18)	27.70 (33.16)	16.18 (19.37)	4.15 (4.96)	28.34 (33.93)	76.37 (91.42)	7.24 (8.66)	6.66 (7.97)	9.33 (11.17)	22.6 (27.0)	9.36	19.0 (22.7)	2.31 (2.76)	5.49 (7.00)	2.76	21.61	4.90
29	15.83	5.06 (6.01)	0.63 (.74)	0.21 (.25)	16.15 (19.18)	27.55 (32.73)	4.77 (5.66)	23.80 (28.27)	72.27 (85.84)	7.82 (9.29)	7.82 (9.29)	11.14 (13.23)	26.0 (30.9)	13.20	20.1 (23.9)	2.50 (2.97)	3.37 (5.76)	3.93	41.47	6.12
35	14.20	3.86 (4.49)	0.83 (.90)	0.31 (.36)	14.84 (17.29)	30.01 (34.98)	4.85 (5.65)	20.56 (23.96)	70.26 (81.88)	5.78 (6.73)	7.71 (9.99)	11.53 (13.43)	25.9 (33.7)	14.19	21.9 (25.5)	2.88 (3.35)	3.37 (5.28)	4.88	36.23	7.27
42	12.35	3.06 (3.51)	1.17 (1.33)	0.64 (.73)	17.25 (19.68)	17.69 (20.18)	5.75 (6.58)	26.20 (29.89)	66.89 (78.31)	6.25 (6.00)	7.92 (9.03)	12.04 (13.72)	35.4 (40.4)	16.19	25.6 (29.2)	3.49 (3.98)	3.69 (4.99)	6.55	25.98	9.51
49	10.12	1.95 (2.17)	1.77 (2.01)	1.06 (1.18)	13.21 (14.69)	14.27 (15.87)	6.39 (7.11)	27.37 (30.45)	61.24 (68.12)	4.93 (5.45)	8.29 (9.22)	12.78 (14.22)	39.0 (43.4)	15.66	23.6 (27.8)	5.10 (5.67)	3.20 (3.76)	9.91	14.91	12.94
56	10.29	1.97 (1.97)	2.23 (2.23)	1.22 (1.30)	16.06 (16.06)	13.18 (14.09)	5.90 (6.57)	25.68 (26.62)	59.17 (65.94)	4.76 (5.30)	12.64 (13.20)	20.25 (21.09)	36.8 (41.0)	14.33	27.8 (31.0)	5.93 (6.61)	3.09 (3.70)	10.89	16.40	14.17
63	8.66	1.59 (1.74)	2.45 (2.45)	1.32 (1.32)	17.98 (19.68)	10.48 (11.57)	4.93 (5.39)	25.29 (27.09)	58.68 (64.22)	4.90 (5.36)	12.06 (13.06)	19.22 (20.57)	37.4 (40.9)	13.68	28.7 (31.0)	6.80 (7.44)	3.18 (3.55)	11.62	9.22	14.71
70	9.40	1.52 (1.67)	2.40 (2.70)	1.22 (1.22)	14.33 (14.33)	10.40 (10.40)	4.67 (5.15)	26.44 (28.18)	53.60 (59.14)	5.92 (6.53)	13.56 (14.96)	21.49 (23.71)	48.2 (53.7)	15.48	32.2 (35.5)	6.93 (7.65)	3.25 (3.55)	12.62	8.81	16.24
77	7.77	1.98 (1.98)	2.28 (2.28)	1.42 (1.42)	17.94 (19.45)	8.63 (10.44)	4.55 (4.96)	26.34 (28.53)	58.46 (63.97)	4.95 (5.37)	14.16 (15.35)	22.79 (24.62)	43.1 (46.7)	17.45	30.3 (32.8)	6.97 (7.55)	1.61 (1.86)	14.10	13.64	15.94
84	8.23	1.71 (1.71)	2.24 (2.70)	1.46 (1.59)	14.52 (15.52)	13.31 (14.30)	4.66 (5.07)	25.20 (27.54)	58.69 (63.93)	4.80 (5.19)	13.95 (15.19)	22.62 (24.64)	40.8 (44.4)	14.17	30.9 (33.7)	7.34 (7.99)	1.24 (1.46)	14.72	15.35	16.20
86	7.49	1.59 (1.59)	2.39 (2.91)	1.69 (1.82)	13.54 (14.63)	12.76 (13.79)	5.07 (5.06)	27.15 (29.34)	56.25 (60.81)	4.42 (4.78)	14.70 (15.99)	23.87 (25.80)	41.4 (44.7)	13.84	31.6 (34.1)	7.74 (8.36)	1.00 (1.12)	15.18	11.02	16.31

¹ Results calculated on the original unextracted plant material.² Corrected for furfural from uronic acids.³ Ash-free basis.⁴ This sample was a composite of plants that were 1, 2, 3, 4, 5, 6, and 7 days old, respectively.⁵ This sample was a composite of plants that were 8, 9, 10, 11, 12, 13, and 14 days old, respectively.

Methoxyl in pure lignin.—The percentage of methoxyl in the lignin residue was determined, and the result was calculated on the basis of ash-free and crude protein-free ($N \times 6.25$) lignin. It is necessary to emphasize in this connection that the term "pure lignin" is used merely to designate the lignin residue from which proper deductions were made for ash and crude protein, and should not be confused with the term as applied to crystalline organic compounds.

RESULTS

The results obtained are given in 3 tables and illustrated graphically in 6 figures. Table 1 shows the percentage composition of barley plants at different stages of growth.

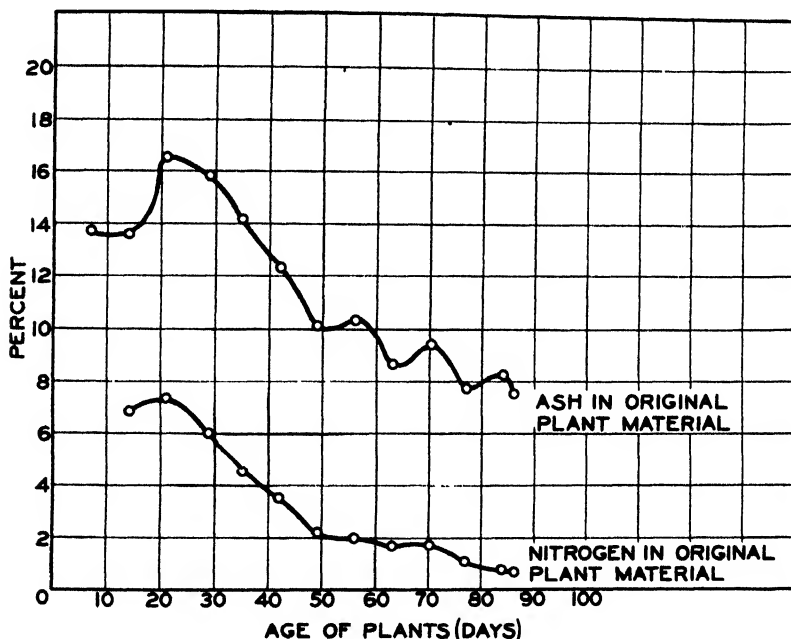


FIGURE 1.—Percentages of ash and nitrogen in leaves and stalks of barley plants at successive stages of growth. (The nitrogen values are on ash-free basis.)

After an initial rise in the percentage of ash, which reached a maximum of 16.48 percent in the third sampling period, there was a progressive although somewhat irregular decrease as the plants grew older (fig. 1). This is in agreement with the results obtained by Lawes and Gilbert (17). Results of a similar nature were obtained with wheat plants by Phillips, Davidson, and Weihe (27), and by Malhotra (18, 19). Norman (21), working with barley plants, also obtained results of a similar character.

NITROGEN

The percentage of nitrogen also showed an increase in the early stages of growth, after which there was a steady decrease (fig. 1). The data obtained by Shaw and Wright (32) in their studies on the

percentage of nitrogen in the corn plant at different stages of growth showed a similar tendency. Phillips, Davidson, and Weihe (27) found a similar decline in the percentage of nitrogen in plants at different stages of growth.

METHOXYL IN ORIGINAL PLANT MATERIAL

In determining the percentage of methoxyl in a plant material by the Zeisel method (26), one obtains not only the percentage of methoxyl found in methyl ethers, but also that present in esterlike combination, as in pectins and in the methyl esters of organic acids generally. The figures listed under this heading represent, therefore, the total methoxyl content of the plant material, that is to say, the

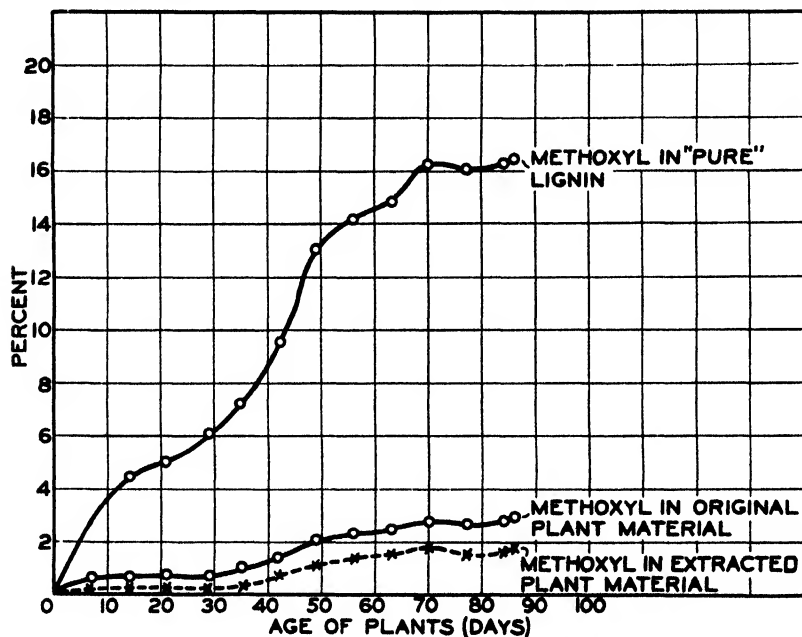


FIGURE 2.—Percentages of methoxyl in original plant material, methoxyl in extracted plant material, and methoxyl in "pure" lignin (all on ash-free basis) in leaves and stalks of barley plants at successive stages of growth.

sum of the percentage of methoxyl present either in the form of methyl ethers or methyl esters. In the early stages of growth the percentage of methoxyl in the plant material was fairly constant (fig. 2). Subsequently there was a progressive rise, presumably caused by the process of lignification, which is accelerated as the plant grows older. The results obtained by Phillips, Davidson, and Weihe (27) with wheat plants show a similar trend, although the percentages of methoxyl in the culms of the mature wheat plants were higher than those recorded in table 1.

METHOXYL IN EXTRACTED PLANT MATERIAL

The percentages of methoxyl in extracted plant material recorded in table 1 represent essentially methoxyl groups present as methyl ethers. The percentages of methoxyl recorded here represent es-

essentially lignin methoxyl groups, although not entirely, as will be pointed out later. It will be noted that in this case, as in the methoxyl in original plant material, the percentage of methoxyl increases with the age of the plant (fig. 2). It will be noted also that the percentage of lignin likewise increases as the plant grows older, so that, on the supposition that the methoxyl in the extracted plate material represents essentially lignin methoxyl, the results are what one would expect.

ALCOHOL-BENZENE EXTRACTIVES

The alcohol-benzene extractives include a heterogeneous class of compounds, as the solvent employed removes various resinous, fatty, or waxy substances. The results show that there was a decrease in the percentage of alcohol-benzene extractives as the plants grew older. From a percentage of more than 30 in the second sampling period, it decreased to 13.54 percent in the last sampling period. The decrease, however, was neither even nor regular.

COLD- AND HOT-WATER EXTRACTIVES

After a slight decrease in the second and third sampling periods, the percentage of cold-water extractives increased, reaching a maximum in the fifth sampling period. The percentage then declined rapidly, although not in a regular manner. The percentage of hot-water extractives at first declined from 6.65 (calculated on ash-free material) to 4.96, then it increased until it reached a maximum of 7.11 in the seventh sampling period. Subsequently, however, it declined again, so that in the last sampling period the percentage amounted to only 3.05.

1-PERCENT HYDROCHLORIC-ACID EXTRACTIVES

The data on the 1-percent hydrochloric-acid extractives are irregular, partly owing to the heterogeneous nature of the extract, as the treatment with acid hydrolyzes some of the hemicelluloses, the polyuronides, the pentosan fraction of the Cross and Bevan cellulose, and perhaps some of the proteins.

TOTAL EXTRACTIVES

The figures presented under this heading represent the sum of the percentages of alcohol-benzene, cold water, hot water, and 1-percent hydrochloric acid extractives. The percentage of total extractives decreased as the plant grew older. There was a slight increase in the second sample, as compared with the first. Subsequently, there was a gradual decrease in the percentage. Attention is called to the fact that, particularly in the early stages of the development of the plant, approximately 80 percent of the plant substance can be removed by successive extraction with 1:2 alcohol-benzene solution, cold water, hot water, and hot 1-percent hydrochloric acid.

URONIC ACIDS

The percentage of uronic acids (calculated as anhydrides) increased during the early development of the plant and reached a maximum when the plants were 29 days old (fig. 3). After that there was a gradual, though not altogether regular, decrease in the percentage of uronic acids. These acids are found chiefly in two structural com-

ponents of the plant, namely, the pectins and the hemicelluloses. As the plants grow older, the pectin content, particularly of lignified tissues, decreases, so that the uronic acids in the plant would naturally show a decrease. Moreover, the pectins contain a much greater percentage of uronic acids than do the hemicelluloses, so that the decrease in pectin would account for a much greater loss in percentage of uronic acids than an equal loss in the hemicelluloses. Attention is called again to the observation of Candlin and Schryver (3, p. 376):

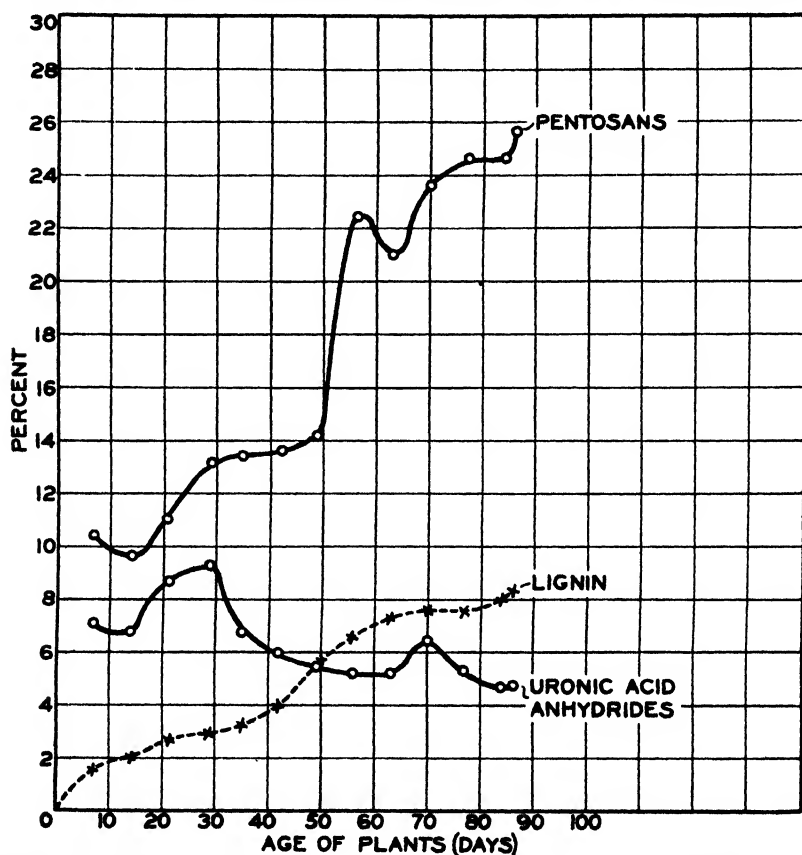


FIGURE 3.—Percentages of uronic acid anhydrides, pentosans, and lignin in leaves and stalks of barley plants at successive stages of growth.

Lignified tissues contain lignins and hemicelluloses in relatively large amounts, with only traces (if any) of pectins. Non-lignified tissues, on the other hand, contain relatively large amounts of pectins, small amounts of hemicelluloses, and no lignin.

TOTAL FURFURAL AND PENTOSANS

The furfural obtained when a plant material is distilled with 12-percent hydrochloric acid is derived from two groups, namely, the pentoses or pentosans, and the uronic acids. These two groups are found in the gums, hemicelluloses, and pectins. A pentose or pentosan unit is also associated with the cellulose in the Cross and Bevan cellulose. The figures on the total furfural in the leaves and stalks

of the barley plants represent, therefore, the sum of the percentages of furfural yielded by the uronic acids and the pentose or pentosan units. The percentage of furfural increased with the increase in the age of the plant.

The figures on the percentage of pentosans were obtained by deducting from the percentage of total furfural the percentage of furfural arising from the uronic acids (the uronic acids yield one-sixth of their weight of furfural) and calculating the difference as pentosans. These figures represent, therefore, the total pentose or pentosan units in the plant material, irrespective of whether these units occur in the gums, hemicelluloses, pectins, or in Cross and Bevan cellulose. Table 1 and figure 3 show that while in the second sampling period

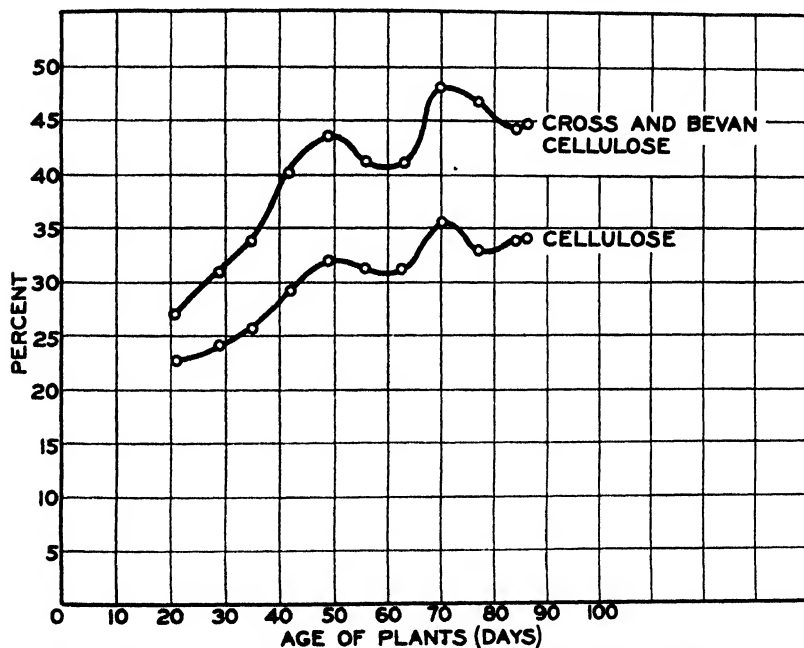


FIGURE 4.—Percentages of Cross and Bevan cellulose and cellulose in leaves and stalks of barley plants at successive stages of growth.

there was a small decrease in the percentage of pentosans as compared with the material from the first sampling period, the plants from the subsequent samplings, with one exception, showed a steady increase until maturity, when the highest percentage was obtained.

CROSS AND BEVAN CELLULOSE, FURFURAL IN CROSS AND BEVAN CELLULOSE, AND CELLULOSE

The percentage of Cross and Bevan cellulose, as well as the percentage of cellulose, increased rapidly as the plant grew older (fig. 4). The maximum percentage was reached when the plants were 70 days old. After this, as the plants matured, the percentages of Cross and Bevan cellulose, as well as cellulose, decreased slightly. When taken in conjunction with the data on the lignin content at various stages of growth of the plant, these data are interesting. As both the

percentage of cellulose and the percentage of lignin increased as the plants grew older, there is no direct evidence that the lignin increased at the expense of the cellulose.

The percentages of furfural in the Cross and Bevan cellulose increased as the plants grew older. The increase, however, was neither consistent nor even. The maximum percentage was reached when the plants were 77 days old, after which there was a decrease, and at maturity the percentage amounted to 13.84, as compared with 17.45 when the plants were 77 days old.

LIGNIN, NITROGEN, ASH, AND METHOXYL IN LIGNIN

Table 1 and figure 3 show that the lignin increased consistently as the plants grew older. In the first sample the percentage of lignin, calculated on the organic portion of the plant material, amounted to 1.71, and in the last sample, when the plants were mature, the percentage of lignin was 8.36. The lignin values obtained in this investigation are quite different from those recorded by Norman (21), although the tendency was the same. According to the results of this investigator, the young seedlings contained more than 14 percent of lignin. The percentage of lignin then increased until a month before maturity, when it amounted to 19.7, after which there was a slight fall. The decided difference between the lignin values recorded in this paper and those reported by Norman is due to the fact that different methods were employed for the quantitative estimation of lignin, Norman using the old method of Ost and Wilkening (24). While none of the methods now employed for the quantitative estimation of lignin is entirely free from criticism, the method of Ost and Wilkening is particularly objectionable, as the residue obtained when the plant material is hydrolyzed with 72 percent sulphuric acid is assumed to be lignin. This is now known to be incorrect, as certain fatty or waxy substances and their degradation products are not removed by the treatment with 72 percent sulphuric acid, and are weighed along with the lignin. Moreover, as was shown by Paloheimo (25), and recently confirmed by Hilpert and Littmann (13), certain carbohydrates when treated with 72 percent sulphuric acid yield insoluble huminlike products. These complexes, therefore, would also be weighed along with the lignin. Furthermore, according to the method of Ost and Wilkening, no correction is applied for the nitrogenous complexes with which the lignin residue is nearly always contaminated. While the percentage of nitrogen in the lignin residue of wood is generally small, this is not the case in the lignin residue from young plants. Attention is called to the nitrogen values in the crude lignin recorded in table 1 and shown graphically in figure 5. The percentage of nitrogen in the lignin residue from the first sample amounted to nearly 7. As the plant grew older, the percentage of nitrogen in the lignin residue decreased, until in the lignin from the mature plant it amounted to only 1. No doubt this was due to the general diminution in the nitrogen content of the leaves and stalks

as the plant grew older. (See figures in table 1 on percentage of nitrogen in original plant material.) The high lignin values obtained when the Ost and Wilkening method is used for the determination of lignin in such materials as young and mature barley plants undoubtedly are due to a contamination of the crude lignin residue with nitrogenous complexes, with huminlike products derived from certain carbohydrates, and with fatty or waxy products and their degradation products. Norman recognized the unsatisfactory character of the method he employed for the determination of lignin, and he states that "the figures are presented with some reserve."

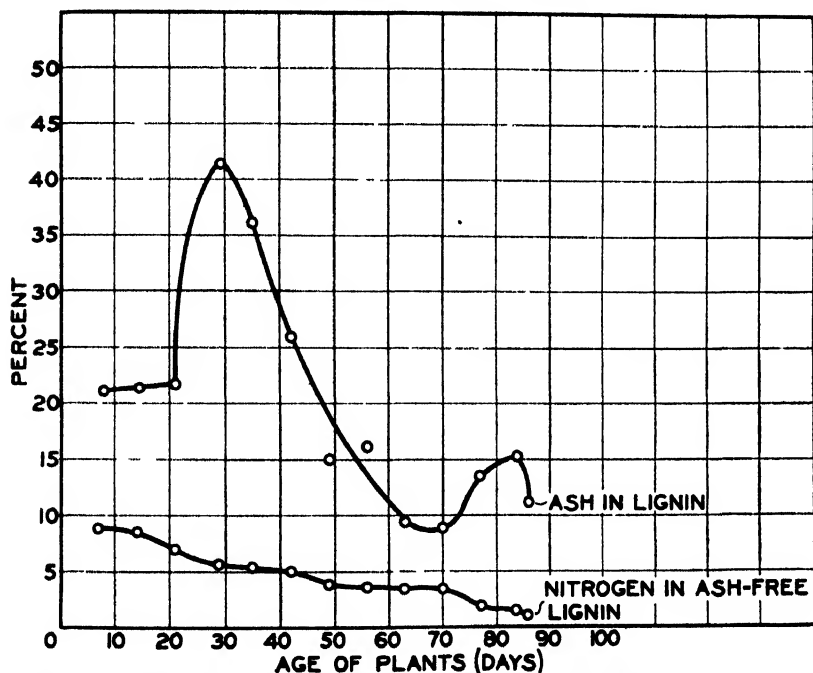


FIGURE 5.— Percentages of ash in lignin and nitrogen in ash-free lignin isolated from leaves and stalks of barley plants at successive stages of growth.

The figures on the percentages of methoxyl in the ash-free lignin and in the so-called "pure" lignin (fig. 2) indicate a decided difference in the composition of lignin, at least so far as its degree of methylation is concerned, isolated from plants at different stages of development. As the plant grows older, not only does the percentage of lignin increase, but there is also a steady increase in the percentage of methoxyl in the lignin.

The percentage of ash in the lignin varied considerably. After an initial increase it decreased, but not consistently (fig. 5).

TABLE 2.—Percentage of furfural yielded by several components of barley plants at successive stages of growth

[Results calculated on basis of ash-free plant material]

Age of plants (days)	Total furfural	Furfural from uronic acids		Furfural from pen- tose of Cross and Bevan cellulose		Furfural from pen- toses of polyu- ronides ¹		Furfural from pentoses of poly- uronides calcu- lated as xylose
		Percent	Percent of total	Percent	Percent of total	Percent	Percent of total	
7 ²	7.31	1.19	16.2					
14 ³	6.78	1.13	16.6					
21	7.97	1.44	18.0	2.52	31.6	4.01	50.4	0.96
29	9.29	1.54	16.5	4.08	43.9	3.67	39.6	6.37
35	8.99	1.12	12.5	4.78	53.2	3.09	34.3	5.33
42	9.03	1.00	11.0	6.54	72.4	1.49	16.6	2.58
49	9.22	.91	9.7	6.80	73.7	1.51	16.4	2.62
56	14.09	.88	6.2	5.87	41.6	7.34	52.1	12.74
63	13.20	.89	6.8	5.59	42.7	6.72	50.5	11.47
70	14.96	1.09	7.2	7.46	49.8	6.41	43.0	11.12
77	15.35	.89	5.8	8.15	53.0	6.31	41.2	10.95
84	15.19	.78	5.1	6.29	41.4	8.12	53.5	14.09
86	15.99	.79	4.9	6.19	38.7	9.01	56.4	15.64

¹ By difference.² This sample was a composite of plants that were 1, 2, 3, 4, 5, 6, and 7 days old, respectively.³ This sample was a composite of plants that were 8, 9, 10, 11, 12, 13, and 14 days old, respectively.

Table 2 shows the percentages of furfural yielded by several components of the leaves and stalks of the barley plants at successive stages of growth (fig. 6). Instead of calculating the percentage of total furfural as pentosans in the conventional manner, an attempt was made to separate the percentages of furfural yielded by the several components of the plant material, thus affording a more accurate picture of the development of the furfural-yielding components. The figures in the second column are taken from Table 1 and are inserted here for the sake of completeness and comparison. The figures in the third column were obtained by dividing the percentages of uronic anhydrides by 6 (the uronic acid anhydrides yield one-sixth of their weight of furfural). The figures in the fifth column were obtained by multiplying in each case the percentage of Cross and Bevan cellulose by the percentage of furfural in the Cross and Bevan cellulose, and dividing the result by 100. The figures in the seventh column were obtained in each case by subtracting the sum of the percentages recorded in the third and fifth columns from the percentage of total furfural given in the second column. The figures given in the last column were calculated from those recorded in the seventh column, taking into consideration the fact that xylose yields only 90 percent of the theoretical quantity of furfural. Table 2 and figure 6 show that after a slight initial increase the percentage of furfural yielded by the uronic acids tended to decrease as the plants matured. The percentage of furfural yielded by the Cross and Bevan cellulose showed an upward trend, with a small decrease in the last two sampling periods. In general, the results indicated that the percentage of furfural-yielding fraction of the Cross and Bevan cellulose increased as the plants matured, and in the later stage of the development (77 days), it furnished more than 50 percent of the total furfural. The percentage of furfural due to the pentoses of the polyuronides showed a decrease in the first few weeks of the development of the

plants, and then it suddenly increased to such an extent that at maturity 56 percent of the total furfural was furnished by the pentoses of the polyuronides. The figures in the last column, of course, show the same tendency as those recorded in the seventh column.

Data are presented in table 3 on the distribution of the firmly bound methoxyl groups between the lignin and the nonlignin constituents of the barley plants at successive stages of growth. The table shows that the weight of lignin methoxyl in 100 g of plant material increased consistently as the plant matured, but the percentage of lignin methoxyl calculated on the basis of the total firmly bound methoxyl did not increase in a regular fashion, although the figures show an upward trend. In the mature plants, from 75 to 80 percent of the total firmly bound methoxyl is found in the lignin.

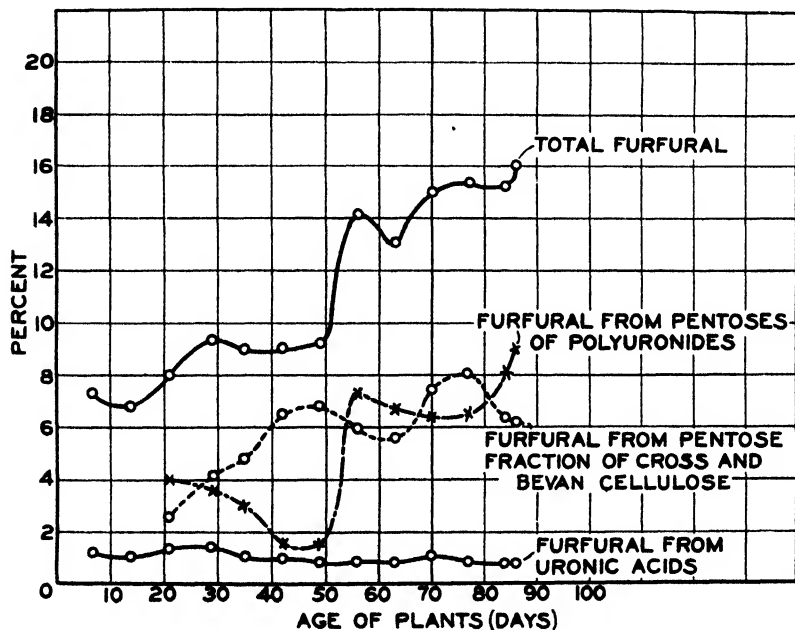


FIGURE 6.—Percentages of furfural yielded by several components of leaves and stalks of barley plants at successive stages of growth.

The percentage of methoxyl not in the lignin decreased, in the main, as the plant matured. In the early stages of the development of the plant, the percentage of methoxyl not in the lignin seemed rather high. It has been shown by O'Dwyer (23), Schmidt and his co-workers (30), and Hägglund and Sandelin (12) that in wood some firmly bound methoxyl groups are found also in the carbohydrate fraction, although the percentages recorded by these investigators are lower. Whether the firmly bound methoxyl obtained from the nonlignin constituents of the plant material represents real methoxyl groups or methyl groups attached directly to carbon which have been split out by the hydriodic acid employed in the determination of methoxyl is not known. The nature of the substance or substances yielding the firmly bound methoxyl not associated with the lignin merits further investigation.

TABLE 3.—*Distribution of firmly bound methoxyl between lignin and nonlignin constituents of barley plants at successive stages of growth*

[Results calculated on basis of ash-free plant material]

Age of plants (days)	Firmly bound methoxyl in 100 g of plant material	Lignin methoxyl in 100 g of plant material	Lignin methoxyl	Methoxyl not in lignin
	Grams	Grams	Percent of total	Percent of total
7 ¹	0.16			
14 ²	.16	0.09	56.2	43.8
21	.18	.13	72.2	27.8
29	.25	.18	72.0	28.0
35	.36	.24	66.6	33.4
42	.73	.38	52.0	48.0
49	1.18	.73	61.8	38.2
56	1.36	.94	69.1	30.9
63	1.82	1.09	71.7	28.0
70	1.72	1.24	72.0	28.1
77	1.64	1.20	77.9	22.9
84	1.59	1.29	81.1	18.3
86	1.82	1.36	74.7	25.3

¹ This sample was a composite of plants that were 1, 2, 3, 4, 5, 6, and 7 days old, respectively.² This sample was a composite of plants that were 8, 9, 10, 11, 12, 13, and 14 days old, respectively.

DISCUSSION

Cross and Bevan (4) advance the following theory of lignification (4, pp. 180-181):

* * * the process of lignification consists in a series of progressive and intrinsic modifications of a cellulose or oxycellulose tissue, the products of modification remaining associated with the residues of the parent substance in a state of combination or of intimate mixture, the final products of metabolism (aromatic products, pentosans, &c.) being excreted and taking no further part in the organic processes of the tissue.

Among others, they advance the following argument in favor of their theory of lignification (4, p. 179):

Regarding lignification as a process of continuous modification of cellulose, and the woods as representing the extreme limits of such a process, these should show an increase in lignone at the expense of cellulose; which is in fact the case. Lignocelluloses in the first year of growth contain 70-80 p. ct. cellulose; the woods, on the other hand, 50-60 p. ct.

While it is true that woods generally contain a greater percentage of lignin than is found in annual plants, it is by no means certain that the evidence offered by Cross and Bevan is an indication that the plant synthesizes lignin from cellulose. The results of this investigation show that the period in which lignin increased was also that in which a considerable increase was obtained in the cellulose. Both the lignin and the cellulose increased progressively with the age of the plant. No direct evidence was obtained that the lignin increased at the expense of the cellulose. If the Cross and Bevan conception is correct, in consideration of the results obtained in this investigation, the assumption would have to be made that the plant synthesizes cellulose faster than it converts it into lignin.

With reference to the hypothesis of Rassow and Zschenderlein (29) that lignin is formed by the plant from pentoses or pentosans, the results obtained in this investigation fail to show any direct relationship between the content of pentose material and lignin. Both the percentage of lignin and the percentage of pentoses increased as the plant matured, and here also, if the hypothesis of Rassow and Zschenderlein is correct, it would be necessary to assume that the plant synthesizes pentoses faster than it converts them into lignin.

The results obtained in this investigation reveal that the percentages of uronic acids and soluble sugars (cold- and hot-water extractives) decreased as the plant matured. In view of the fact that the total pentose material increased with the age of the plant and that soluble pentoses are also included among the cold- and hot-water extractives, the loss of soluble carbohydrates other than pentoses must have been even greater than the figures on the cold- and hot-water extractives reveal. It is, of course, recognized that much of this loss was due to a translocation of the hexoses to the seed where it was stored in the form of starch; it is nevertheless conceivable that part of the hexose sugars may have been utilized by the plant in the production of lignin. Whether the synthesis of lignin proceeds directly or whether the hexoses are first oxidized to uronic acids is, of course, not known. While the results obtained in this investigation do not disprove the hypotheses of Cross and Bevan and of Rassow and Zschenderlein, they definitely do not support them. In the main, the results are more in harmony with Schrauth's conception as to the genesis of lignin in the plant.

SUMMARY

A study was made of the composition of the leaves and stalks of the barley plant at successive stages of growth.

After an initial increase, the percentages of ash and nitrogen declined steadily as the plant matured.

The percentage of methoxyl in the original and in the extracted plant materials increased with the age of the plant.

The percentages of alcohol-benzene, cold-water, and hot-water extractives declined, though not in a regular manner, as the plant became older. The 1-percent hydrochloric acid extractives showed no definite tendency.

The percentage of uronic acids increased somewhat during the early development of the plant, and then declined as the plant matured. The percentage of the furfural-yielding components, as well as the percentage of pentoses calculated as pentosans, increased as the plant matured. After a slight initial increase, the percentage of furfural yielded by the uronic acids tended to decrease, and in no case did it amount to more than 18 percent of the total. The percentage of furfural furnished by the Cross and Bevan cellulose showed an upward trend at first, but decreased somewhat as the plants matured. The furfural derived from the pentoses of the polyuronides showed a decrease in the first few weeks of the development of the plants, and then suddenly increased to such an extent that at maturity 56 percent of the total furfural was furnished by the pentoses of the polyuronides.

The percentages of Cross and Bevan cellulose, as well as the cellulose, increased rapidly as the plants grew older. As the plants matured there was a slight decrease in the percentage of these two components.

The percentage of lignin, as well as the methoxyl in the lignin, increased in a regular manner as the plants developed and matured. The lignin from young plants differs from the lignin of mature plants in that the former contains a much smaller percentage of methoxyl. As the plants mature not only does the percentage of lignin greatly

increase, but there is also a rapid methylation of the hydroxyl groups of the lignin.

In the mature plants, 75 to 80 percent of the firmly bound methoxyl groups are found in the lignin.

No direct evidence was obtained that the barley plant synthesizes lignin from cellulose, pentoses, or pentosans. The results obtained are more in harmony with the hypothesis that the barley plant synthesizes lignin from soluble sugars other than pentoses.

LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Compiled by the committee on editing methods of analysis. Ed. 3, 593 pp., illus. Washington, D. C.
- (2) BECKMANN, E., LIESCHE, O., and LEHMANN, F.
1923. QUALITATIVE UND QUANTITATIVE UNTERSCHIEDE DER LIGNINE EINIGER HOLZ-SPUND STROHARTEN. *Biochem. Ztschr.* 139: [491]-508, illus.
- (3) CANDLIN, E. J. and SCHRYVER, S. B.
1928. INVESTIGATIONS OF THE CELL-WALL SUBSTANCES OF PLANTS, WITH SPECIAL REFERENCE TO THE CHEMICAL CHANGES TAKING PLACE DURING LIGNIFICATION. *Roy. Soc. [London], Proc., Ser. B* 103: 365-376, illus.
- (4) CROSS, C. F., BEVAN, E. J., and BEADLE, C.
1916. CELLULOSE. AN OUTLINE OF THE CHEMISTRY OF THE STRUCTURAL ELEMENTS OF PLANTS WITH REFERENCE TO THEIR NATURAL HISTORY AND INDUSTRIAL USES. New ed., 328 pp., illus. London, New York, [etc.]
- (5) DAVIDSON, J., and PHILLIPS, M.
1930. LIGNIN AS A POSSIBLE FACTOR IN LODGING OF CEREALS. *Science* (n. s.) 72: 401-402.
- (6) DICKSON, A. D., OTTERSON, H., and LINK, K. P.
1930. A METHOD FOR THE DETERMINATION OF URONIC ACIDS. *Jour. Amer. Chem. Soc.* 52: 775-779, illus.
- (7) DUSTMAN, R. B., and SHRIVER, L. C.
1931. THE CHEMICAL COMPOSITION OF AMBROSIA TRIFIDA AT SUCCESSIVE GROWTH STAGES. *Jour. Amer. Soc. Agron.* 23: 190-194.
- (8) EHRLICH, F.
1930. UEBER DIE CHEMIE DES PEKTINS UND SEINE BEZIEHUNGEN ZUR BILDUNG DER INKRUSTEN DER CELLULOSE. *Cellulosechemie* 11. 161-170.
- (9) EULER, A. C., VON.
1921-22. UEBER DEM LIGNIN NAHESTEHENDE HARZE UND GERBSÄUREN DER FICHTENNADELN. EIN BEITRAG ZUR KENNTNIS DES LIGNINS. *Cellulosechemie* 2: 128-135, 1921; 3: 1-7, 1922.
- (10) FUCHS, W.
1927. THEORIE DER LIGNIN-BILDUNG. *Biochem. Ztschr.* 180: [30]-34.
- (11) ———
1928. ZUR PHYSIKALISCHEN STRUKTUR DES FICHTEN-LIGNINS. *Biochem. Ztschr.* 192: [165]-166.
- (12) HÄGGLUND, D., and SANDELIN, O.
1934. UEBER DEN AZETYL-SPUND METHOXYLGEHALT DES FICHTENHOLZES. *Papier Fabrikant* 32: 253-255. (In section Verein der Zellstoff- und Papier-Chemiker u.—Ingenieure.)
- (13) HILPERT, R. S., and LITTMANN, E.
1934. ÜBER DIE VERHÄRZUNG DER ZUCKER DURCH SÄUREN UND IHRE BEZIEHUNG ZUR LIGNIN-BESTIMMUNG. *Ber. Deut. Chem. Gesell.* 67: 1551-1556.
- (14) KEDZIE, R. C.
1893. I. COMPOSITION OF WHEAT AT DIFFERENT PERIODS OF RIPENING; OF THE STRAW AT THE SAME PERIODS. II. COMPOSITION OF CERTAIN FORAGE PLANTS: SPURRY: LATHYRUS SILVESTRIS: MINT HAY. III. ADULTERATION OF GROUND FEED. IV. MINERAL RESIDUES IN SPRAYED FRUIT. *Mich. Agr. Expt. Sta. Bull.* 101, 21 pp., illus.

- (15) KLASON, P.
1917. BIDRAG TILL KÄNNEDOMEN OM GRANVEDLIGNINETS KEMISKA BYGGNAD. Arkiv Kemi, Min. och Geol. Bd. 6, no. 15, 21 pp.
- (16) KÖNIG, J., and RUMP, E.
1914. CHEMIE UND STRUKTUR DER PFLANZEN-ZELLMEMBRAN. 88 pp., illus. Berlin.
- (17) LAWES, SIR J. B., and GILBERT, J. H.
1884. ON THE COMPOSITION OF THE ASH OF WHEAT-GRAIN, AND WHEAT-STRAW GROWN AT ROTHAMSTED, IN DIFFERENT SEASONS, AND BY DIFFERENT MANURES. Jour. Chem. Soc. [London] 45: 305-407.
- (18) MALHOTRA, R. C.
1932. THE DISTRIBUTION OF SOME RESERVE SUBSTANCES IN HARD WINTER WHEAT PLANT AT SUCCESSIVE GROWTH STAGES AND THEIR POSSIBLE UTILISATION. Jour. Agr. Sci. [England] 22: [485]-496, illus.
- (19) ———
1933. A CONTRIBUTION TO THE BIOCHEMISTRY OF THE WHEAT PLANT. Jour. Biochem. Tokyo 18: 199-205, illus.
- (20) NORMAN, A. G.
1929. THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS. I. THE NATURE AND QUANTITY OF THE FURFURALDEHYDE-YIELDING SUBSTANCES IN STRAWS. Biochem. Jour. 23: [1353]-1366.
- (21) ———
1933. A PRELIMINARY INVESTIGATION OF THE DEVELOPMENT OF STRUCTURAL CONSTITUENTS IN THE BARLEY PLANT. Jour. Agr. Sci. [England] 23: [216]-227, illus.
- (22) ODÉN, S.
1926. OM LIGNINETS UPPKOMST OCH OMVANDLING I VÄXTRIKET. Svensk Kem. Tidskr. 38: 122-130.
- (23) O'DWYER, M. H.
1928. PRELIMINARY INVESTIGATIONS ON THE CONSTITUTION OF THE HEMICELLULOSES OF TIMBER. Biochem. Jour. 22: [381]-390.
- (24) OST, H., and WILKENING, L.
1910. DIE VERZUCKERUNG DES ZELLSTOFFS. Chem. Ztg. 34: [461]-462.
- (25) PALOHEIMO, L.
1929. BEITRÄGE ZUR LIGNINBESTIMMUNG MIT SÄUREHYDROLYSE. Biochem. Ztschr. 214: [161]-174, illus.
- (26) PHILLIPS, M.
1932. THE QUANTITATIVE DETERMINATION OF METHOXYL, LIGNIN, AND CELLULOSE IN PLANT MATERIALS. Jour. Assoc. Off. Agr. Chem. 15: 118-131, illus.
- (27) ——— DAVIDSON J., and WEIHE, H. D.
1931. STUDIES OF LIGNIN IN WHEAT STRAW WITH REFERENCE TO LODGING. Jour. Agr. Research 43: 619-626.
- (28) ——— GOSS, M. J., and BROWNE, C. A.
1933. DETERMINATION OF URONIC ACIDS AND METHOXYL IN CERTAIN PLANTS AND PLANT MATERIALS. Jour. Assoc. Off. Agr. Chem. 16: 289-292.
- (29) RASSOW, B., and ZSCHENDERLEIN, A.
1921. ÜBER DIE NATUR DES HOLZES DES HANFES. Ztschr. Angew. Chem. 34: 204-206.
- (30) SCHMIDT, E., MEINEL, K., JANDEBEUR, W., and SIMSON, W.
1932. DIE KETTENLÄNGE DER CELLULOSEN NATIVER ZUSAMMENSETZUNG UND DIE KETTENLÄNGE DES ACETYL-XYLANS DER LAUBHÖLZER. Cellulosechemie 13: 129-139.
- (31) SCHRAUTH, W.
1923. ÜBER DAS LIGNIN. Ztschr. Angew. Chem. 36: 149-152.
- (32) SHAW, R. H., and WRIGHT, P. A.
1921. A COMPARATIVE STUDY OF THE COMPOSITION OF THE SUNFLOWER AND CORN PLANTS AT DIFFERENT STAGES OF GROWTH. Jour. Agr. Research 20: 787-793.
- (33) VER HULST, J. H., PETERSON, W. H., and FRED, E. B.
1923. DISTRIBUTION OF PENTOSANS IN THE CORN PLANT AT VARIOUS STAGES OF GROWTH. Jour. Agr. Research 23: 655-663.

THE DISTRIBUTION AND CONDITION OF PHOSPHORUS IN THREE HORIZONS OF A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN METAL CYLINDERS¹

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INTRODUCTION

The writer has recently reported (47)² the distribution and condition of the nitrogen in the soil during an experiment with apple trees grown in metal cylinders for a period of 6½ years, and which received annually for the last 3 years of growth different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate. The distribution and condition of the phosphorus is reported in the present paper. The principal objective was to determine the condition of the residual phosphoric acid (P_2O_5) derived from the added monocalcium phosphate. 98.7 and 87.3 percent of the phosphoric acid was still present in the surface 0 to 7 inches of the NPK-treated cylinders under sod and cultivation, respectively, at the end of the experiment.

The mechanism of the absorption by soils of certain anions (phosphate, tartrate, oxalate, and citrate) is comparable in certain respects to that of cations, that is, it is one of exchange involving equilibrium (8, 9, 10). The characteristics of these exchange reactions are that they take place in the boundary between two phases, and that consequently no distinction can be made between absorption, adsorption, and chemical reaction in this interface. All such reactions may be described as disperse reactions (50).

The physicochemical examination of the system phosphate, ferric hydroxide, aluminum hydroxide, calcium (and magnesium) hydroxide by Gaarder (21) has thrown considerable light on the course of the reactions that might occur under soil conditions, albeit the experiments were in vitro, i. e., they were not conducted with soils present. These experiments of Gaarder show that the systems resulting depend on the concentrations of hydrogen, iron, aluminum, calcium, and magnesium ions relative to the phosphate ions. When the conditions are such that the concentrations of iron, aluminum, and calcium are in excess of that of the phosphate only two narrow pH ranges were observed at which the concentrations of phosphate (PO_4) were more than 0.01 mg per liter, viz, at 3.9 to 4.5 and 6.7 to 7.3.

The approach to the problem of the condition of the residual phosphate applied in the writer's cylinder experiments has been made by ascertaining the total amount of phosphorus present in the three horizons, 0 to 7, 7 to 21, and 21 to 53 inches, respectively, of the soils of the treated and untreated cylinders, together with the determination of the amounts extracted from these soils under specified conditions by solvents having a wide range of pH values.

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² Reference is made by number (italics) to Literature Cited, p. 336.

METHODS OF EXPERIMENTATION

FIELD PLAN OF THE CYLINDER EXPERIMENTS

The detailed plan of this experiment has appeared in a number of publications (2, 45, 46); only a brief outline, therefore, is necessary.

The soil used in the cylinders is a virgin soil of Trenton formation formed by the weathering of limestone (44). In the present paper this soil is designated the "original soil." The excavation was made on a strip of land 110 feet by 11 feet near the college experimental orchard. The surface horizon is a heavy silt loam and is underlain by a clay loam which becomes heavier in texture with depth (44). During the excavation the three horizons, viz, surface (0 to 7 inches), subsurface (7 to 21 inches), and subsoil (21 to 53 inches), were kept separate and each pile was thoroughly mixed. The procedure of filling the boiler-plate cylinders, which were 5 feet in diameter and 5½ feet deep, has been previously described (47).

The trees were planted in the spring of 1922. The culture system, consisting of green manuring with buckwheat and rye principally, was uniform in all the cylinders until the spring of 1924, at which time half the cylinders were seeded with a mixture of Kentucky bluegrass and timothy. These cylinders are designated "cylinders under sod." The remaining half of the cylinders were kept under a system of clean cultivation. These are designated "cylinders under cultivation."

A distinction must be noted with respect to the additions of phosphoric acid from 1925 until the end of the experiment in 1927. During these last 3 years of the experiment the cylinders under cultivation received 2.5 g more phosphoric acid than the cylinders under sod. The reason for this is that it was then considered necessary to add equal amounts of organic matter to all the cylinders under cultivation. This was accomplished by growing rye outside the cylinders. For further details the paper by Anthony and Clarke (2, p. 251) should be consulted. All trees were allowed to grow without the addition of any mineral fertilizer until the spring of 1925, at which time differential treatment with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate was commenced.

The schedule of applications of monocalcium phosphate is given in table 1.

The total amount of phosphorus (as P_2O_5) added during the experiment was 1,052.8 g. In addition to the phosphorus carried in the monocalcium phosphate the cover crops contributed approximately 10 g of phosphoric acid. The fertilizer was broadcasted and not mixed with the soil.

TABLE 1.—Schedule of monocalcium phosphate applications with phosphorus pentoxide equivalent

Date applied	$CaH_4(PO_4)_2 \cdot H_2O$	P_2O_5 equivalent
	Grams	Grams
Apr. 18, 1925.....	534	300.8
May 3, 1926.....	267	150.4
June 7, 1926.....	267	150.4
May 6, 1927.....	534	300.8
May 18, 1927.....	267	150.4
Total.....	1,869	1,052.8

The total precipitation during the period of the experiment was 94.4 inches and, in addition, 2 inches of artificial watering was applied in May 1926, and 1 inch in August 1927.

Samples of the soil of each of the horizons were taken both before and after the completion of the experiment in the manner previously described (47). During the period from September 20 to 28, 1927, the trees were dug up and soil samples representative of the three horizons were taken, by the method of successive quartering, from each of the cylinders from which the trees had been removed. These samples were dried at 75° C. and then sieved through a 1-mm sieve (3) and stored in glass jars in the dark. Analyses of the trees have already been reported (46).

PERCOLATION EXPERIMENTS

CHOICE OF SOLVENTS

The solvent powers of a particular acid, barring reverse or secondary reactions, are related not only to the extent of dissociation of the salts formed and to the dissociation constant of the acid but also to the extent of hydrolysis and to complex ion formation. Moreover, it must be borne in mind that the determination by the aid of weak acids of so-called "available" phosphoric acid (P_2O_5) is not one of dissolution pure and simple but is a mechanism of exchange involving equilibrium (8, 11).

With respect to the choice of solvents one might, as some investigators have done (4, 27, 28), use only one acid, e. g., hydrochloric acid of different concentrations, thus providing a wide range of pH values. It was decided to use different acids in the present investigation not only because the different solvents chosen provide a wide range of pH values (0.7 to 5.5), but also because they have long been in use by other investigators, and thus permit a comparison of the results obtained with those of others. That fundamental differences with respect to their ability to mobilize phosphoric acid exist is indicated by the difference in their critical concentrations (10).

The choice of an acid does not appear to be altogether an arbitrary one for all types of soils, as assumed by some investigators. Laterite soils which are very high in iron oxides are decomposed by hydroxy acids with the formation of acetone and carbon dioxide (7), and some hydroxy acids, e. g., citric acid, apparently fail with certain types of calcareous soils (6), possibly as a result of a too great reduction of the acidity by the calcium carbonate.

The majority of chemical determinations of the availability of phosphate have been carried out by the "equilibrium" method. The solvents used have included principally (1) citric acid, used by Dyer (13); (2) the 0.2 normal nitric acid solvent used extensively by Fraps and his coworkers (16, 17); (3) 0.1 or 0.2 normal acetic acid solution, used in France and Germany; and (4) the solvent recently proposed by Truog (48), viz, a 0.002 normal sulphuric acid solution buffered with ammonium sulphate. Distilled water has been favored by Schloesing (40) and by Wrangell (52, 53, 54).

EXTRACTION PROCEDURE

Extraction methods in which the soil is shaken for a definite length of time with the solvent, followed by filtration, washing, and repetition of the procedure with the soil residue, are inconvenient and laborious. Percolation methods which give the same kind of results as the foregoing procedure are much more convenient. In one form or another this method has been used by many investigators. Recently Harper (25) has described a simple form of apparatus which has been further modified by the writer, as follows:

A carbon tube is fitted with a platinum cone, above which rests a layer of filter-paper pulp, followed by a layer of 40-mesh leached

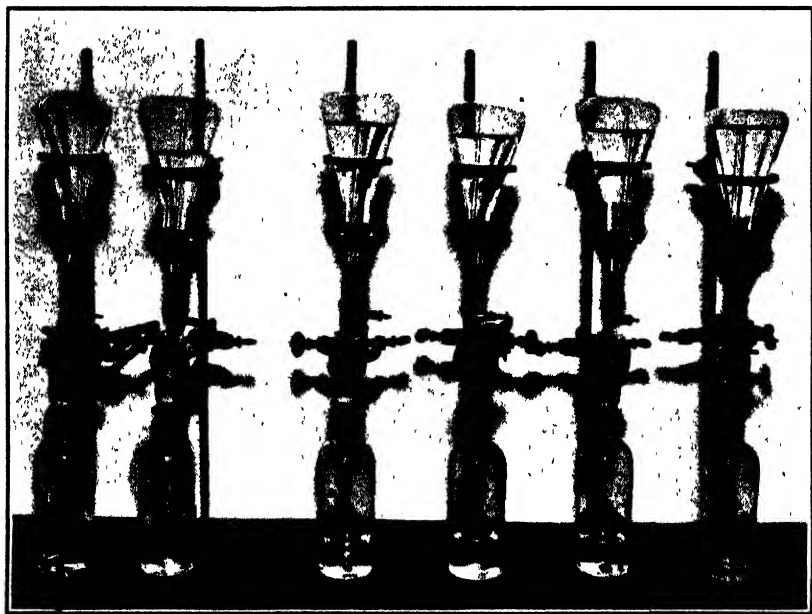


FIGURE 1.—Battery of the type of percolation apparatus used in the extraction procedure.

quartz sand. Five grams of the soil (moisture-free basis) are then added, gently compacted by tapping, and a layer of 60-mesh acid-washed quartz sand is added. The reservoir consists of a 250-cc flask fitted with air inlet and outlet tube to which is attached a piece of heavy walled rubber tubing fitted with a screw cock. This cock should work freely and be well greased. A battery of such percolation apparatus (fig. 1) is very compact and utilizes little laboratory space. The principle of the method used is one of solution and displacement, and the mechanism is similar to that described by Parker (34). The action of a dilute acid on a soil is not constant (38), and for this reason the rate of flow can be regulated sufficiently uniformly to give agreement between duplicates by making slight adjustments of the screw cock morning and evening. The extractions were made at laboratory temperature, 75° to 85° F.

DETERMINATION OF PHOSPHORIC ACID IN THE EXTRACTS

The four dilute acids used were 0.1 normal acetic (pH 2.8), 0.2 normal nitric (pH 0.7), 0.173 normal citric (pH 2.2), and 0.002 normal sulphuric buffered with ammonium sulphate (pH 3.0). Distilled water (pH 5.5) not freed from carbon dioxide was also used.

The determination of phosphorus in the distilled water and also in the acetic and sulphuric acid leachates was made by the Denigès method (12), and in the citric acid and nitric acid leachates by Richards and Godden's modification (37) of the Pemberton-Neumann method (33, 35). In the latter procedure soluble silica was previously removed by dehydration.

The blue color of the Denigès method is the result of a partial reduction of some of the phosphomolybdate. Within certain limits the color is proportional to the concentration of phosphorus in the solution. The method is very sensitive; 0.001 mg phosphoric acid in 100 cc solution can easily be detected and estimated. The literature relating to the method has been thoroughly reviewed by Zinzadze (55), who has developed a technic whereby the blue color is stable over a long period.

EXPERIMENTAL DATA

The quantities in parts per million and absolute (total) amounts of phosphoric acid in the different soil horizons are given in table 2. The results are the mean of duplicate determinations of total phosphoric acid (3), which differed by less than 0.005 percent. The results of the percolation experiments with water, sulphuric, acetic, citric, and nitric acids are given in table 3. The volume of each leachate in all cases was 200 cc.

TABLE 2.—*Parts per million and absolute amounts of total phosphoric acid in the respective horizons before the trees were planted and at the end of the experiment*

Treatment	Total phosphoric acid			Absolute amount total phosphoric acid			
	Surface (0-7 inches)	Subsurface (7-21 inches)	Subsoil (21-53 inches)	Surface (0-7 inches)	Subsurface (7-21 inches)	Subsoil (21-53 inches)	Total (0-53 inches)
Soil before trees were planted.....	<i>P. p. m.</i> 1,020	<i>P. p. m.</i> 950	<i>P. p. m.</i> 1,020	<i>Grams</i> 597	<i>Grams</i> 1,120	<i>Grams</i> 2,744	<i>Grams</i> 4,461
Sod.							
Check.....	1,017	948	1,016	595	1,118	2,733	4,446
NPK.....	2,796	939	1,017	1,636	1,107	2,736	5,479
PK.....	2,804	948	1,018	1,641	1,118	2,738	5,497
P.....	2,819	954	1,018	1,650	1,125	2,738	5,513
Cultivation.							
Check.....	1,028	955	1,013	602	1,127	2,727	4,456
NPK.....	2,587	1,045	1,015	1,514	1,232	2,732	5,478
PK.....	2,623	1,032	1,018	1,535	1,217	2,738	5,490
P.....	2,645	1,030	1,018	1,548	1,215	2,738	5,501

TABLE 3.—Phosphoric acid in successive leachings with distilled water, and with sulphuric, acetic, citric, and nitric acids, expressed in parts per million and in absolute amounts

DISTILLED WATER (pH 5.5)

Description of soil	Phosphoric acid in successive leaching indicated															Total P_2O_5 in all leachings	Absolute amounts
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Soil before trees were planted:	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i> ¹	Grams
0-7 inches	0	0														0	5.0
7-21 inches	0	0														0	6.2
21-53 inches	0	0														0	6.7
Check (sod):																	
0-7 inches	8.7	(*)														8.7	5.0
7-21 inches	5.3	0														5.3	6.2
21-53 inches	2.5	0														2.5	6.7
Check (cultivation):																	
0-7 inches	10.2	0														10.2	6.0
7-21 inches	5.3	0														5.3	6.3
21-53 inches	2.5	0														2.5	6.7
NPK (sod):																	
0-7 inches	229.0	109.9	77.8	48.1	37.8	28.6	22.9	18.3	17.2	13.7	10.3	9.2	8.9	6.9	5.5	644.1	375.9
7-21 inches	5.9	0														5.9	6.9
21-53 inches	2.7	0														2.7	7.3
NPK (cultivation):																	
0-7 inches	194.5	87.5	56.2	40.3	33.7	22.3	17.1	13.8	11.9	9.8	8.7	7.6	6.5	5.9	5.1	521.2	304.9
7-21 inches	6.8	0														6.8	8.0
21-53 inches	2.7	0														2.7	7.3

¹ Expressed in terms of the dry weight of soil.

* Trace.

0.002 N SULPHURIC ACID BUFFERED WITH AMMONIUM SULPHATE (pH 3.0)

Description of soil	Phosphoric acid in successive leaching indicated										Total P ₂ O ₅ in all leach- ings	Absolute amounts
	1	2	3	4	5	6	7	8	9	10		
Soil before trees were planted:	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m. ¹	Grams
	0	0	0	0	0	0	0	0	0	0	0	0
	0-7 inches	0	0	0	0	0	0	0	0	0	0	0
	7-21 inches	0	0	0	0	0	0	0	0	0	0	0
Check (soil):	0-7 inches	13.3	7.1	2.7	0	0	0	0	0	0	23.1	13.5
	7-21 inches	8.7	4.6	3.2	0	0	0	0	0	0	16.5	19.5
	21-33 inches	3.0	0	0	0	0	0	0	0	0	3.0	8.1
	0-7 inches	0	0	0	0	0	0	0	0	0	0	0
Check (cultivation):	0-7 inches	17.8	12.5	7.2	0	0	0	0	0	0	37.5	21.9
	7-21 inches	8.9	4.2	0	0	0	0	0	0	0	13.1	15.4
	21-33 inches	3.0	0	0	0	0	0	0	0	0	3.0	8.1
	0-7 inches	0	0	0	0	0	0	0	0	0	0	0
NPK (soil):	0-7 inches	230.8	343.5	230.8	76.0	34.1	6.9	6.4	2.3	0	930.8	544.6
	7-21 inches	9.4	4.1	0	0	0	0	0	0	0	13.5	15.8
	21-33 inches	6.4	0	0	0	0	0	0	0	0	6.4	17.2
	0-7 inches	0	0	0	0	0	0	0	0	0	0	0
NPK (cultivation):	0-7 inches	234.0	278.2	231.0	109.2	44.2	8.0	6.4	2.2	0	913.2	534.3
	7-21 inches	11.4	9.4	5.7	5.1	0	0	0	0	0	31.6	37.3
	21-33 inches	6.4	0	0	0	0	0	0	0	0	6.4	17.2
	0-7 inches	0	0	0	0	0	0	0	0	0	0	0

¹ Expressed in terms of the dry weight of soil.² Trace.

TABLE 3.—*Phosphoric acid in successive leachings with distilled water, and with sulphuric, acetic, citric, and nitric acids, expressed in parts per million and in absolute amounts—Continued*

0.1 N ACETIC ACID (pH 2.8)

Description of soil	Phosphoric acid in successive leaching indicated																				Total P ₂ O ₅ in all leachings	Absolute amounts
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	P. p. m.	P. p. m.
Soil before trees were planted:	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
0-7 inches	11.9	4.4	1.1	0																	17.4	10.2
7-21 inches	3.4	1.1	(1)	0																	4.5	5.3
21-53 inches	3.2	1.1	(1)	0																	4.3	11.6
Check (sod):																						
0-7 inches	15.6	6.6	1.1	0																	23.3	13.6
7-21 inches	4.6	4.4	1.1	0																	10.1	11.9
21-53 inches	3.2	2.7	1.1	0																	7.0	13.8
NPK (sod):																						
0-7 inches	357.2	87.0	50.4	50.4	52.7	45.8	41.2	32.1	22.9	14.7	14.0	13.7	12.4	11.5	9.8	8.2	7.3	5.5	4.6	2.3	843.7	493.7
7-21 inches	3.9	1.1	0																		5.0	5.9
21-53 inches	3.7	1.1	0																		4.8	12.9

¹ Expressed in terms of dry weight of soil.

² Traces.

1 PERCENT CITRIC ACID (pH 2.2)

Description of soil	Phosphoric acid in successive leaching indicated										Total P ₂ O ₅ in all leach- ings	Absolute amounts		
	1	2	3	4	5	6	7	8	9	10				
Soil before trees were planted:	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	Grams	
	0-7 inches.....	125.4	53.2	39.9	20.9	19.0	17.5	16.2	14.7	13.2	13.0	333.0	194.8	
	7-21 inches.....	22.6	13.0	(1)								35.5	41.9	
	21-63 inches.....	20.0	8.0	(1)								28.0	75.3	
	Check (sod):													
	0-7 inches.....	138.0	72.5	45.4	38.2	33.6	30.5	26.3	18.0	10.0	8.0	420.5	246.0	
	7-21 inches.....	17.4	7.1	0	0							24.5	28.9	
	21-63 inches.....	21.0	9.8	5.6	0							36.4	97.9	
	Check (cultivation):													
	0-7 inches.....	142.0	85.6	52.1	33.8	30.5							63.6	
NPK (sod):	7-21 inches.....	28.2	15.3	7.9	2.5	0						37.8	101.7	
	21-63 inches.....	21.0	10.0	6.8	0							53.9	63.6	
	0-7 inches.....	1,096.3	176.7	119.7	87.4	68.4	60.8	60.8	55.0	38.0	22.8	1,785.9	1,045.0	
	7-21 inches.....	21.4	10.0	6.2	3.7	0						41.3	48.7	
	21-63 inches.....	21.0	11.0	6.0	0							38.0	102.2	
	NPK (cultivation):													
	0-7 inches.....	902.4	138.7	90.6	70.2	53.2	51.4	45.5	40.4	38.7	25.4	1,456.5	852.2	
	7-21 inches.....	38.7	24.3	21.1	20.4	10.2	6.2	0				120.9	142.6	
	21-63 inches.....	19.0	10.2	7.1	(1)							36.3	97.6	

0.2 N NITRIC ACID (pH 0.7)

Soil before trees were planted:												
0-7 inches	56.0	62.6	45.6	30.4	32.2	30.4	22.4	20.0	19.1	18.0	336.7	197.0
7-21 inches	13.0	8.0	(1)								21.0	24.8
21-53 inches	13.2	8.0	(2)								23.2	62.4
Check (sod)												
0-7 inches	64.6	60.8	57.0	49.4	45.6	38.0	32.3	30.4	30.4	22.8	431.3	252.4
7-21 inches	10.0	7.8	(1)								16.8	19.8
21-53 inches	10.0	7.5	(2)								17.5	47.1
NPK (sod)												
0-7 inches	1,178.0	135.0	91.2	60.8	41.8	39.9	22.9	20.4	20.4	17.1	1,627.5	952.3
7-21 inches	25.2	7.2	(1)								32.4	38.2
21-53 inches	24.7	7.8	(2)								32.5	87.4

1 Expressed in dry weight of soil.

2 Trace.

3 Percolation was not carried further.

DISCUSSION AND INTERPRETATION OF RESULTS

EXTENT OF AND DIFFERENCES IN THE DOWNWARD MOVEMENT OF PHOSPHORUS IN THE TWO CULTURE SYSTEMS AS DETERMINED BY TOTAL PHOSPHORUS CONTENT

The weights of each of the three soil horizons are: Surface 1,290 pounds, subsurface 2,600 pounds, and subsoil 5,930 pounds. The absolute amounts of phosphoric acid can, therefore, be calculated. They are shown in table 2.

The absolute amounts of phosphoric acid in the surface soil of correspondingly treated cylinders under the two systems is very much greater under sod than under cultivation, but the condition is reversed in the subsurface layer. The quantities of residual applied phosphoric acid expected in the surface soil at the end of the experiment can be approximately calculated from the data of table 2 together with the known amounts applied and absorbed by the trees from the added phosphate (46). The data so calculated indicate that a downward movement of phosphorus into the subsurface occurred in all the cylinders under cultivation and that no movement into the subsurface occurred under sod. The absolute amounts of phosphoric acid which moved into the subsurface (7 to 21 inches) layer in the cylinders under cultivation were: NPK, 117 g; PK, 113 g; and P, 99 g—equivalent to 11.5 and 9.5 percent of the amounts added in the NPK- and P-treated cylinders.

The difference in the behavior of the cylinders under cultivation with respect to phosphorus movement as well as with respect to the movement of nitrogen (47) is interesting. For a better understanding it will be necessary to refer to the distribution and condition of the nitrogen in these same cylinders (47). In order to explain the accretion of nitrogen as nonnitric nitrogen in the subsoil (21 to 53 inches) under cultivation (no accretion of nitrogen occurred under sod), three explanations were advanced, viz, (1) assimilation of applied nitric nitrogen by micro-organisms, (2) peptization of organic nitrogen by sodium nitrate in the surface soil and subsequent movement into the subsoil, and (3) the greater root system of the apple trees under cultivation as compared with that under sod.

In searching for an explanation of this movement of phosphorus (and also of nitrogen) in the cylinders under cultivation (but not from under sod) the first explanation given above would a priori be eliminated. The third explanation would account for only a fraction of the phosphoric acid that moved into the subsurface. An additional explanation has recently been suggested, viz, that the living grass roots themselves absorbed and held enough phosphorus as it became available to reduce the amount that moved downward to a negligible quantity. This would be a consequence of greater downward movement of water under cultivation than under sod because there would be less transpiration and, therefore, less upward movement. These causes, no doubt, would be a factor but a minor factor because the phosphoric acid content of the grass in the sod cylinders would account for less than one twenty-fifth of the phosphoric acid that moved into the subsurface layer of the cylinders under the cultivation system.

The second explanation above, viz, that of "peptization" by the organic matter, remains to be considered. There is sufficient experimental evidence (29, 30, 36) to show that colloiddally dispersed humic

acids (formed by bacterial activity) function in making the soil phosphoric acid more soluble. Ramann (36) gives many instances of soils being impoverished in phosphorus under such conditions. Dicalcium phosphate (CaHPO_4) is relatively quickly decomposed by humic acids with the formation of calcium humates and free phosphoric acid, the fate of which depends on the amount of sesquioxides present (30). The action of the humic acids may be expressed as a "deactivation" of the sesquioxides (9, 10).

The relative immobility of phosphorous in the cylinders under sod is in accord with the results obtained in many other long-continued field experiments on the heavier types of soils (14, pp. 12-124; 43, 49), where little movement of applied phosphates occurred below plow depth, except where certain salts (14) or stable manures (43) were added. In orchards under sod, penetration of phosphates in heavy clay soils may not exceed the first inch (42). On the other hand, on loosely compacted soils evidence of phosphate movement below the first foot has been obtained (42, 43, 51). Phosphoric acid has not been found in the leachates from lysimeter experiments (22, 31). In prairie soils a gradual translocation of phosphorus from the sub-surface to the surface horizons has been noted (1).

CONDITION OF THE PHOSPHORUS

SOURCE OF THE PHOSPHORUS AND OF THE IRON AND ALUMINUM IN THE HAGERSTOWN SERIES

Frear and White (19) in an examination of the available phosphoric acid on the grasslands of the Jordan fertilizer plots found only 6.4 percent of the total amount of phosphoric acid present soluble by the Dyer method (one extraction only), and observed that this indicated the absence of apatite. The very small amounts of phosphoric acid dissolved from the original soil by all solvents (table 3) point to the absence of apatite. This is further confirmed by the mineralogical examination of Honess (44, footnote 2). Limonite comprises two-thirds of the accessory species in the fine sand fraction (0.2 to 0.1 mm) of the surface soil, and it is probable that the source of phosphorus in this soil is as an impurity in the limonite ($\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$), known to contain admixtures of other elements including phosphorus (5). Limonite has a high "fixing" capacity for phosphorus (15).

During the weathering processes aluminum compounds are in part transferred into various hydrated silicates. The aluminum of this soil is derived from the feldspars, which constitute about 50 percent of the fine-sand fraction and a large proportion of the silt fraction. Other sources of aluminum are the amphiboles and pyroxenes present as accessory species. There is no evidence of the presence of bauxite in this soil (44). The iron is present as iron oxides mostly as limonite.

PERCOLATION DATA

The leaching experiments were limited to an examination of (1) the original soil before planting (the pH of which was 6.8), (2) the check cylinders under sod and cultivation (the pH of which was 6.65 and 6.60, respectively), (3) the NPK cylinders also under sod and cultivation (the pH of which was 6.50).

The results of the percolation experiments for the three horizons of the foregoing soils are given in table 3.

CONDITION OF THE RESIDUAL PHOSPHORUS IN THE SURFACE HORIZONS

In the case of those solvents having a pH value of 2.2 and below the concentration of the solution with respect to phosphoric acid falls with each successive extraction, and with all solvents tends toward a constant level. At the stage indicated by reduction to this constant level there must exist a phosphate of such low solubility that the amount going into solution at each extraction is independent of the mass present in the soil. But with none of the solvents do the amounts going into solution follow a logarithmic law of decrement in either the phosphate-treated or the untreated soils—a consequence of the existence of more than one phosphate in both soils. In the phosphate-treated soils nitric and citric acids have brought out 60 to 70 percent of the total phosphoric acid in the surface 0 to 7 inches in the first extract, indicating the presence of at least one comparatively easily soluble phosphate which dissolves in proportion to the mass of it present in the soil.

Inasmuch as there is no evidence in these laboratory experiments that the added monocalcium phosphate has effected any significant changes in the solubility of the native phosphorus, we can proceed to ascertain the condition of the added phosphate by comparing the amounts removed by the different solvents after an equal number (ten) of leachings, allowance being made for the amounts extracted from the check cylinder by the respective solvents. The amount dissolved from the unfertilized soil (i. e., the check cylinder) must be taken into consideration because the quantities of phosphoric acid removed by different acids from the check cylinder may bear (as in this experiment) no constant relation to that extracted from the fertilized soil. The results for the NPK cylinder in sod are shown in table 4.

TABLE 4.—Phosphoric acid (P_2O_5) dissolved from the surface soil of the NPK cylinders expressed as a percentage of the residua of the amount applied

IN SOD

Solvent	Indicated method of calculation	Percentage of residua applied P_2O_5 dissolved
Distilled water.....	$\frac{353.1-5.0}{1,039} \times 100 =$	33.5
0.1 N acetic acid.....	$\frac{441.4-13.6}{1,039} \times 100 =$	41.2
0.002 N H_2SO_4	$\frac{544.6-13.5}{1,039} \times 100 =$	51.1
0.2 N HNO_3	$\frac{952.2-252.4}{1,039} \times 100 =$	67.3
0.17 N citric acid.....	$\frac{1,045.0-246.0}{1,039} \times 100 =$	76.9

IN CULTIVATION

Distilled water.....	$\frac{285.2-6.0}{917} \times 100 =$	30.4
0.002 N H_2SO_4	$\frac{534.3-21.9}{917} \times 100 =$	55.9
0.17 N citric acid.....	$\frac{852.2-246.0}{917} \times 100 =$	66.1

The action of a dilute acid upon soil is not a simple action of attack of an easily soluble phosphate followed by a more difficultly soluble phosphate (23, 24), nor, indeed, as already pointed out, is the action of a dilute acid constant (38). Rather does extraction with the different weak acids result in a condition of equilibrium which modifies the initial partition of phosphoric acid in the solid and liquid phases. If the amount of phosphoric acid in the liquid phase is sufficiently high the solution will give up phosphoric acid to the soil, and vice versa. There must exist, therefore, a critical concentration (9); this critical concentration is different for different acids, low for acetic and nitric acids and high for citric acid (10). This critical concentration depends on the phosphorus reserves of the soil and also on the "fixing" power. It is also clear from Russell and Prescott's (38) experiments that no definite fraction of the phosphates present is obtained by extraction with dilute acids but only that part expressed as the difference between the amount dissolved and absorbed. For all these reasons it is not possible by such means to separate each of the different phosphorus compounds—to separate, for example, the phosphates of calcium from one another—by treatment of such an adsorption substrate as a soil with different dilute acids, as some investigators have attempted to do.

One may, however, from the data of table 4 conclude that one-third of the residual applied phosphoric acid from the NPK cylinder in sod remained (under conditions of continuous leaching) in a condition soluble in water, one-third has been transformed into somewhat less soluble compounds, and one-third (100–67 percent) into very difficultly soluble compounds.

The results for the surface 0 to 7 inches of the phosphate-treated cylinder under cultivation are of the same order of magnitude for the distilled water and sulphuric-acid extractions, viz, 30.4 and 55.9 percent, respectively. But the percentage of the amount residual in the surface layer under cultivation dissolved by citric acid is only 66.1 compared with 76.9 from the correspondingly treated cylinder under sod. This apparently anomalous result will be reserved for later discussion.

The first and second distilled water percolates of the surface 0 to 7 inches of the treated cylinders, both in the sod and in the cultivation systems, contain as much as 13.5 and 7.9 percent of the residual amounts applied during the course of this experiment. This fact suggests that the amounts added may have been more than sufficient to saturate the aluminum and iron hydroxides in these surface layers.

The difficultly soluble compounds into which the soluble phosphate has in part been transformed must be more difficultly soluble than chemically pure ferric phosphate. This may be deduced from the results given in table 5, in which are shown the results of percolation experiments in which chemically pure ferric phosphate, aluminum phosphate, and tricalcium phosphate were each mixed with the original soil in amounts equal to the application of phosphoric acid to the phosphate-treated cylinders. Table 5 gives the percentages of phosphoric acid removed by the different solvents.

TABLE 5.—Phosphoric acid (P_2O_5) removed by the percolation method from c. p. ferric phosphate, c. p. aluminum phosphate, and c. p. calcium phosphate mixed with the "original" soil

Solvent	pH	$FePO_4 \cdot 4H_2O$		$AlPO_4$		$Ca_3(PO_4)_2$	
		Extractions	Re-moved	Extractions	Re-moved	Extractions	Re-moved
		Number	Percent	Number	Percent	Number	Percent
Distilled water.....	5.5	10	0	10	14	10	21
0.002 N sulphuric acid.....	3.0	10	0	10	32.6	4	100
0.1 N acetic acid.....	2.8	10	7.9	10	66	6	100
0.17 N citric acid.....	2.2	8	100	10	100	2	100
0.2 N nitric acid.....	0.7	15	100	10	100	2	100

The results are similar to those obtained by Heck (26) and also are in accord with Gaarder's scheme (21). Relative to the comment given on page 333, it may be added that the nearest information that can be obtained with respect to the proportions of the various phosphates existing in the phosphate-treated cylinders is through a comparison of the data of table 5 with those of table 4.

CONDITION OF THE PHOSPHORUS IN THE LOWER HORIZONS

Neither water nor 0.002 N sulphuric acid leached out any phosphoric acid from the original soil. The native phosphorus of these soils is, therefore, not soluble in these solvents. Citric acid removed somewhat more than nitric acid from the lower layers of the original soil.

Percolation with water also removed only minute quantities from the lower horizons of the phosphate-treated (and the untreated) cylinders. One-percent citric acid has dissolved greater quantities of phosphoric acid than 0.2 N nitric acid.

The condition of the phosphoric acid that moved from the surface soil into the subsurface of the phosphate-treated cylinder under cultivation is easily traced. Water removed none of it; Truog's solvent dissolved approximately 20 percent and citric acid about 80 percent of it.

EFFECT OF THE GROWTH OF TREES ON THE SOLUBILITY OF THE NATIVE PHOSPHORUS

More phosphoric acid was dissolved from the surface soil of the check cylinders than from the original soil by all solvents. The increase is very marked with citric and also with nitric acid; the relative increases are 51.2 and 55.4 g, respectively, for the cylinder under sod. Inasmuch as these increments are much greater than the amounts of phosphoric acid contributed in the cover crops (p. 322), the native phosphorus of the soil must have been made more soluble during the growth of the trees.

THE ORGANIC PHOSPHORUS

A number of methods have been proposed for determining the organic phosphorus of soils, but none has yet met the criteria of validity indicated by Frear and White (20) in 1911. In these cylinder experiments the organic phosphorus of even the phosphate-treated plots under cultivation as determined by Schollenberger's method

(41) represented only about 1 percent of that applied as monocalcium phosphate. The organic phosphorus is, therefore, relatively insignificant as compared to the amount of phosphoric acid applied.

THE "FIXING" CAPACITY OF SOILS

The writer has determined the fixing capacity of the original Hagerstown soil by many of the different methods proposed to determine this property (15, 16, 18, 25). It is unnecessary, however, to record the results, inasmuch as no additional information was secured beyond that already recorded—a fact which lends further support to the suggestion (p. 333) that the amounts of phosphate applied during the course of this experiment were more than sufficient to saturate the iron and aluminum hydroxides. The fixing capacity of any one soil type is, as already indicated, a relative and not an absolute property; the amount retained is conditioned not only by the concentration of the phosphate ions and the concentration with respect to other elements, especially those of hydrogen, silica, iron, aluminum, calcium, and magnesium (9, 32), but also by temperature and by time (15, 39, 56). The results for the same soil will, therefore, differ with the laboratory procedure with respect to the latter factors. If the fixing capacity of different soil types is to be compared, the method described by Demolon and Barbier (10) should receive the attention of investigators.

The ability to fix phosphorus cannot be unrelated to the form of the phosphate. Wrangell (52) is probably correct in maintaining that the concentration of the soil solution with respect to phosphate ions depends to a greater extent upon the absorbing capacity of the soil than upon the structure and composition of the applied phosphate, for the soil solution has, in all cases examined by Wrangell, a greater concentration with respect to PO_4 ions when treated with tricalcium phosphate (and also rock phosphate) than with monocalcium phosphate. Deductions, however, relative to the availability of phosphates cannot be made from such observations, for, although relatively insoluble phosphates are fixed more slowly than the more soluble phosphates, they are less efficient in supplying available phosphorus to plants (39)—a consequence apparently of solid phase feeding.

SUMMARY

The distribution of phosphoric acid (P_2O_5) in three horizons at the conclusion of an experiment lasting $6\frac{1}{2}$ years on a Hagerstown clay loam soil, contained in cylinders planted to apple trees, and treated with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate, are given in percentage and in absolute amounts.

In all the phosphate-treated cylinders the total (i. e., absolute) amounts of phosphoric acid of the surface soil was greater in the cylinders under sod than in those under cultivation. But in the subsurface layer (7 to 21 inches) the absolute amounts of phosphoric acid present were considerably smaller in the cylinders under sod than in those under cultivation. The downward movement of phosphoric acid into the subsurface layer in the cylinders under cultivation is equivalent approximately to 10 percent of the phosphoric acid added.

There is no evidence of movement of phosphoric acid into the sub-surface layer in the cylinder under sod and none into the subsoil (21 to 53 inches) of the cylinders either under cultivation or under sod.

An explanation advanced is that the differences in the movement of phosphorus in the two culture systems is for the most part the result of the mobilizing effect of humic acids.

A simple and compact percolation apparatus is described by the use of which the quantities of phosphoric acid removed in successive leachates from the original soil before the trees were planted and in the soils from untreated and phosphate-treated cylinders by various weak acid solvents were obtained. The results of these extractions are as follows:

In the phosphate-treated cylinders at least one moderately soluble phosphate was present which dissolves in proportion to the mass of it in the soil.

Not a trace of phosphoric acid was found in the leachates from any horizon of the original soil (soil before trees were planted) either by distilled water (pH 5.5) or by 0.002 normal sulphuric acid (pH 3.0).

Thirty-three percent of the phosphoric acid applied to the phosphate-treated cylinders was still soluble in distilled water at the end of the 7-year experiment. One-third remained in a condition of moderate solubility and one-third was converted into basic iron and aluminum compounds more difficultly soluble than ferric phosphate.

There was a conversion into difficultly soluble phosphates of that portion of the applied phosphoric acid that had moved into the sub-surface layer in the cylinder under cultivation.

A marked increase was observed in the availability of the phosphoric acid of the soil in the check cylinders over that of the original soil.

LITERATURE CITED

- (1) ALWAY, F. J., and ROST, C. O.
1916. THE VERTICAL DISTRIBUTION OF PHOSPHORUS IN THE SURFACE SOIL OF PRAIRIES. *Soil Sci.* 2: 493-497.
- (2) ANTHONY, R. D., and CLARKE, W. S., JR.
1932. GROWTH RECORD OF FERTILIZED APPLE TREES GROWN IN METAL CYLINDERS. *Jour. Agr. Research* 44: 245-266, illus.
- (3) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Compiled by the committee on editing methods of analysis. Ed. 3, 593 pp., illus. Washington, D. C.
- (4) BOBKO, E.
1932. DIE LÖSLICHKEITSKURVEN DER BODENPHOSPHORSÄURE ALS MITTEL ZUR BESTIMMUNG DER BODENFRUCHTBARKEIT. 2d Internatl. Cong. Soil Sci. (Moscow) Comm. 4: 103-109, illus.
- (5) CLARKE, F. W.
1916. THE DATA OF GEOCHEMISTRY. Ed. 3, U. S. Geol. Survey Bull. 616, 821 pp.
- (6) DAS, S.
1926. THE DETERMINATION OF AVAILABLE PHOSPHORIC ACID OF CALCAREOUS SOILS. . . India Dept. Agr. Mem., Chem. Ser. 8: 69-104, illus.
- (7) DEAN I. A., and DEAN, A. L.
1929. DECOMPOSITION OF CITRIC ACID BY SOIL. *Soil Sci.* 28: 281-287, illus.
- (8) DEMOLON, A.
1933. FIXATION ET ÉCHANGE DE CERTAINS ANIONS PAR LES COLLOIDES DU SOL. *Mezőgazdasági Kutatások 'Sigmund Special Number* 6: 474-479.

- (9) DEMOLON, A., and BARBIER, G.
1929. FIXATION ET MOBILISATION DE P_2O_5 DANS LES LIMONS. *Compt. Rend. Acad. Sci. [Paris]* 189: 1310-1312.
- (10) ——— and BARBIER, G.
1932. LA CONCENTRATION CRITIQUE D'ÉQUILIBRE ET L'APPRECIATION DU BESOIN DES SOLS EN ACIDE PHOSPHORIQUE. 2d Internatl. Cong. Soil Sci. (Moscow) *Comm.* 4: [86]-96, illus.
- (11) ——— and BASTISSE, F.
1933. INFLUENCE DES ANIONS SUR LA FIXATION ET LA MOBILISATION DE L'ACIDE PHOSPHORIQUE DANS LES SOLS. *Compt. Rend. Acad. Sci. [Paris]* 197: 1247-1249.
- (12) DENIGÉS, M. G.
1920. REACTION DE COLORATION EXTRÊMEMENT SENSIBLE DES PHOSPHATE ET DES ARSÉNIATES. SES APPLICATIONS. *Compt. Rend. Acad. Sci. [Paris]* 171: 802-804.
- (13) DYER, B.
1894. ON THE ANALYTICAL DETERMINATION OF PROBABLY AVAILABLE "MINERAL" PLANT FOOD IN SOILS. *Chem. Soc. (London) Trans.* 65: 115-167.
- (14) ———
1902. RESULTS OF INVESTIGATIONS ON THE ROTHAMSTED SOILS . . . U. S. Dept. Agr. Off. Expt. Stas. *Bull.* 106, 180 pp.
- (15) FORD, M. C.
1933. THE NATURE OF PHOSPHATE FIXATION IN SOILS. *Jour. Amer. Soc. Agron.* 25: 134-144.
- (16) FRAPS, G. S.
1910. ACTIVE PHOSPHORIC ACID AND ITS RELATION TO THE NEEDS OF THE SOIL FOR PHOSPHORIC ACID IN POT EXPERIMENTS. *Tex. Agr. Expt. Sta. Bull.* 126, 72 pp., illus.
- (17) ———
1912. THE ACTIVE POTASH OF THE SOIL AND ITS RELATION TO POT EXPERIMENTS. *Tex. Agr. Expt. Sta. Bull.* 145, 39 pp., illus.
- (18) ———
1922. THE FIXATION OF PHOSPHORIC ACID BY THE SOIL. *Tex. Agr. Expt. Sta. Bull.* 304, 22 pp.
- (19) FREAR, W., and WHITE, J. W.
1910. GENERAL COMPOSITION OF THE GRASS LANDS CONTIGUOUS TO THE GENERAL FERTILIZER PLATS. A STUDY UPON A LOWER SILURIAN LIMESTONE SOIL. *Pa. State Col. Ann. Rept.* 1909-10 (pt. 2): 163-235, illus.
- (20) ——— and WHITE, J. W.
1911. THE GENERAL COMPOSITION OF THE GRASS LANDS CONTIGUOUS TO THE GENERAL FERTILIZER PLATS: A STUDY UPON A LOWER SILURIAN LIMESTONE SOIL. THIRD REPORT. THE HUMUS: THE CONDITION OF THE PHOSPHORUS AND SULPHUR. (By J. W. White). CONCLUSIONS FROM THE FOREGOING DATA. (By Frear and White.) *Pa. State Col. Ann. Rept.* 1910 11: 313-348.
- (21) GAARDER, T.
1930. DIE BINDUNG DER PHOSPHORSÄURE IM ERDBODEN. DIE LÖSLICHKEIT DER PHOSPHORSÄURE IN WASSERIGEN ELEKTROLYTLÖSUNGEN BEI WECHSELNDEN pH-WERT UND KATIONEN-INHALT. *Vestlandets Forstl. Forsøkssta. Meddel.* Bd. 4, Hefte 4, 140 pp., illus.
- (22) GERLACH, M.
1931. DIE UMSETZUNG DER WASSERLÖSLICHEN PHOSPHORSÄURE IM BODEN. *Superphosphate* 7: 130-132.
- (23) HALL, A. D., and AMOS, A.
1906. THE DETERMINATION OF AVAILABLE PLANT FOOD IN THE SOIL BY THE USE OF WEAK ACID SOLVENTS. PART II. *Jour. Chem. Soc. (London) Trans.* 89: 205-222, illus.
- (24) ——— and PLYMEN, F. J.
1902. THE DETERMINATION OF THE AVAILABLE PLANT FOOD IN SOILS BY THE USE OF WEAK ACID SOLVENTS. *Jour. Chem. Soc. (London) Trans.* 81: 117-144, illus.

- (25) HARPER, H.
1932. DETERMINATION OF THE EASILY SOLUBLE PHOSPHORUS IN SOILS. *Science* (n. s.) 76: 415-416.
- (26) HECK, A. F.
1934. PHOSPHATE FIXATION AND PENETRATION IN SOILS. *Soil Sci.* 37: 343-355, illus.
- (27) HIBBARD, P. L.
1931. CHEMICAL METHODS FOR ESTIMATING THE AVAILABILITY OF SOIL PHOSPHATE. *Soil Sci.* 31: 437-466, illus.
- (28) ———
1933. ESTIMATION OF PLANT AVAILABLE PHOSPHATE IN SOIL. *Soil Sci.* 35: 17-28.
- (29) JENSEN, C. A.
1917. EFFECT OF DECOMPOSING ORGANIC MATTER ON THE SOLUBILITY OF CERTAIN INORGANIC CONSTITUENTS OF THE SOIL. *Jour. Agr. Research* 9: 253-268.
- (30) KAPPEN, H., and BOLLENBECK, K.
1925. UEBER DIE BEDEUTUNG DER AZIDITÄTSFORMEN DER BÖDEN FÜR DAS LÖSLICHWERDEN SCHWERLÖSLICHER PHOSPHATE. *Ztschr. Pflanzenernähr. Düngung* (A) 4: 1-29.
- (31) LYON, T. L., and BIZZELL, J. A.
1918. LYSIMETER EXPERIMENTS. RECORDS FOR TANKS 1 TO 12 DURING THE YEARS 1910 TO 1914 INCLUSIVE. N. Y. (Cornell) Agr. Expt. Sta. Mem. 12, 115 pp., illus.
- (32) MATTSON, S.
1928. ANIONIC AND CATIONIC ABSORPTION BY SOIL COLLOIDAL MATERIALS BY VARYING $\text{SiO}_2/\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$ RATIO. 1st Internatl. Cong. Soil Sci. (Washington), 1927, Conn. 2: 199-211.
- (33) NEUMANN, A.
1902. EINFACHE VERASCHUNGSMETHODE (SÄUREGEMISCHVERASCHUNG) UND VEREINFACHTE BESTIMMUNGEN VON EISEN, PHOSPHORSÄURE, SALZSÄURE UND ANDEREN ASCHENBESTANDTHEILEN UNTER BENUTZUNG DIESER SÄUREGEMISCH-VERASCHUNG. *Hoppe-Seyler's Ztschr. Physiol. Chem.* 37: [115]-142.
- (34) PARKER, F. W.
1921. METHODS OF STUDYING THE CONCENTRATION AND COMPOSITION OF THE SOIL SOLUTION. *Soil Sci.* 12: 209-232.
- (35) PEMBERTON, H., JR.
1893. THE DETERMINATION OF PHOSPHORIC ACID BY THE TITRATION OF THE YELLOW PRECIPITATE WITH STANDARD ALKALI. *Jour. Amer. Chem. Soc.* 15: 382-395.
- (36) RAMANN, E.
1911. BODENKUNDE. Aufl. 3, ungearb. und verb., 619 pp., illus. Berlin.
- (37) RICHARDS, M. B., and GODDEN, W.
1924. THE PEMBERTON-NEUMANN METHOD FOR THE ESTIMATION OF PHOSPHORUS. *Analyst* 49: 565-572.
- (38) RUSSELL, E. J., and PRESCOTT, J. A.
1916. THE REACTION BETWEEN DILUTE ACIDS AND THE PHOSPHORUS COMPOUNDS OF THE SOIL. *Jour. Agr. Sci. [England]* 8: [65] 110, illus.
- (39) SCARSETH, G. D., and TIDMORE, J. W.
1934. THE FIXATION OF PHOSPHATES BY SOIL COLLOIDS. *Jour. Amer. Soc. Agron.* 26: 138-151, illus.
- (40) SCHLOESING, T.
1870. ANALYSE DES EAUX CONTENUES DANS LES TERRES ARABLES. *Compt. Rend. Acad. Sci. [Paris]* 70: 98-102.
- (41) SCHOLLENBERGER, C. J.
1918. ORGANIC PHOSPHORUS OF SOIL: EXPERIMENTAL WORK ON METHODS FOR EXTRACTION AND DETERMINATION. *Soil Sci.* 6: 365-395.
- (42) SMOCK, R. M., and GOURLEY, J. H.
1932. A SURVEY OF RESIDUA FROM PHOSPHORUS APPLICATIONS ON ORCHARD SOILS. *Amer. Soc. Hort. Sci. Proc.* (1931) 28: 509-514, illus.
- (43) STEPHENSON, R. E., and CHAPMAN, H. D.
1931. PHOSPHATE PENETRATION IN FIELD SOILS. *Jour. Amer. Soc. Agron.* 23: 759-770.

- (44) THOMAS, W.
1923. ULTIMATE ANALYSIS OF THE MINERAL CONSTITUENTS OF A HAGERSTOWN SILTY CLAY LOAM SOIL AND OCCURRENCE IN PLANTS OF SOME OF THE ELEMENTS FOUND. *Soil Sci.* 15: 1-18.
- (45) ———
1932. COMPOSITION OF CURRENT AND PREVIOUS SEASON'S BRANCH GROWTH IN RELATION TO VEGETATIVE AND REPRODUCTIVE RESPONSES IN *PYRUS MALUS* L. *Plant Physiol.* 7: 391-445, illus.
- (46) ———
1933. ABSORPTION, UTILIZATION, AND RECOVERY OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY APPLE TREES GROWN IN CYLINDERS AND SUBJECTED TO DIFFERENTIAL TREATMENT WITH NUTRIENT SALTS. *Jour. Agr. Research* 47: 565-581, illus.
- (47) ———
1934. THE DISTRIBUTION AND CONDITION OF NITROGEN IN THREE HORIZONS OF A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN METAL CYLINDERS. *Jour. Agr. Research* 48: 845-856.
- (48) TRUOG, E.
1930. THE DETERMINATION OF THE READILY AVAILABLE PHOSPHORUS OF SOILS. *Jour. Amer. Soc. Agron.* 22: 874-882.
- (49) VAN ALSTINE, E.
1918. THE MOVEMENT OF PLANT-FOOD WITHIN THE SOIL. *Soil Sci.* 6: 281-308.
- (50) WIEGNER, G.
1918. BODEN UND BODENBILDUNG IN KOLLOIDCHEMISCHER BETRACHTUNG. 98 pp., illus. Dresden and Leipzig.
- (51) WILHELMJ, A., GERICKE, S., and SIEMENS, K. H.
1932. URSACHEN DER WIRKUNG DES THOMASMEHLS. II. DAS VERHALTEN DES THOMASMEHLS IM BODEN. *Phosphorsäure* 2: 257-302.
- (52) WRANGELL, M. V.
1926. I. ÜBER BODENPHOSPHATE UND PHOSPHORSÄURE-BEDÜRFTIGKEIT. *Landw. Jahrb.* 63: [627]-668.
- (53) ———
1926. II. KOLORIMETRISCHE METHODE ZUR SCHNELLEN BESTIMMUNG VON PHOSPHORSÄURE IN SEHR VERDÜNNTEN LÖSUNGEN. *Landw. Jahrb.* 63: [669]-676, illus.
- (54) ——— and KOCH, E.
1926. III. DIE LÖSLICHKEITSGESETZE IN IHRER ANWENDUNG AUF TERTIÄRE PHOSPHATE. *Landw. Jahrb.* 63: [677]-706, illus.
- (55) ZINZADZE, S. R.
1930. NEUE METHODEN ZUR KOLORIMETRISCHEN BESTIMMUNG DER PHOSPHOR- UND ARSENSÄURE. *Ztschr. Pflanzenernähr. Düngung u. Bodenk.* (A) 16: [129]-184.
- (56) ———
1933. STUDIEN ÜBER DIE LÖSLICHKEIT EINIGER IM BODEN VORKOMMENDER PHOSPHATE (RÖNTGENOSKOPISCHE UNTERSUCHUNGEN AN PHOSPHATEN). 2d Internatl. Cong. Soil Sci. (Moscow) 1930 Comm. 2: 179.

A COMPARISON OF LEPTOSPHAERIA SALVINII AND HELMINTHOSPORIUM SIGMOIDEUM IRREGULARE¹

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INTRODUCTION

It has been shown previously (9)² that *Leptosphaeria salvinii* Catt. on rice has a conidial stage, originally described as *Helminthosporium sigmoideum* Cav. (3), and a sclerotial stage, originally described as *Sclerotium oryzae* Catt. (1, 2). This fungus occurs on rice in Arkansas, California, Louisiana, and Texas in the United States and has been reported also from Italy, Japan, India, the Philippine Islands, Bulgaria, China, and Cochinchina.

For the past 3 years the writers have observed a similar yet distinctly different fungus on rice in Arkansas, Louisiana, and Texas. This fungus has a conidial stage which is very similar to *Helminthosporium sigmoideum* and a sclerotial stage which is distinctly different from that originally described by Cattaneo as *Sclerotium oryzae*. As no perithecial stage has been observed for the fungus it has been described as *H. sigmoideum* var. *irregulare* (4). It was described as a variety rather than as an independent species because of the similarity of the conidia to those of *H. sigmoideum*. The differences are chiefly in the sclerotia.

The symptoms and seasonal development of the disease caused by *Helminthosporium sigmoideum irregulare* are similar to those of the stem rot caused by *Leptosphaeria salvinii* as previously described (9, 10) except that in most instances the severity of the disease caused by the former fungus is not so great as that caused by the latter. It is the purpose of the present paper to describe the new fungus more adequately and to compare it with the corresponding stages of *L. salvinii* as previously described.

DESCRIPTION OF HELMINTHOSPORIUM SIGMOIDEUM VAR. IRREGULARE CRALLEY AND TULLIS

Mycelium.—Hyphae white to olivaceous, septate, profusely branched, 2 μ to 5 μ in diameter. In culture, aerial mycelium usually scanty, submerged mycelium dark. On host, mycelium scanty. Numerous appressoria produced at times.

Sclerotial stage.—Sclerotia very numerous, irregular in outline, black, surface rough, 90 μ to 119 μ by 268 μ to 342 μ . Habitat, leaf sheaths and culms of rice, *Oryza sativa* L.

Conidial stage.—Conidiophores dark-colored, septate, erect, simple, 4 μ to 5 μ by 75 μ to 200 μ ; conidia borne singly on sharp-pointed sterigmata, fusiform, typically three-septate, frequently with germ tubes 2 or 3 times the length of the spore on spores still attached to the conidiophore, intercalary cells densely granular, terminal cells less granular than intercalary cells, 9 μ to 12 μ by 41 μ to 58 μ . Habitat, leaf sheaths of rice and on sclerotia floating on water.

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² Reference is made by number (italic) to Literature Cited, p. 348.

COMPARATIVE MORPHOLOGY OF *LEPTOSPHAERIA SALVINII* AND *HELMINTHOSPORIUM SIGMOIDEUM IRREGULARE*

Cultures of *Helminthosporium sigmoideum irregulare* from rice in the United States, Japan,³ and the Philippine Islands⁴ have been studied and all found to be essentially the same. Conidia occurred in all cultures but most abundantly in those from Louisiana and Arkansas in the United States and from Saga, Japan. The sclerotial stage of this fungus also has been reported by Park and Bertus in Ceylon (6), and a similar, or perhaps identical, fungus has been described in Japan by Sakurai (7) as *Sclerotium* no. 3.

The sclerotia of *Leptosphaeria salvinii* (*Sclerotium oryzae*) were originally described by Cattaneo (1) as being spherical and measuring 350μ to 400μ in diameter. Specimens of cotype material⁵ in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture, shown in figure 1, A, B, have been examined, and the material agrees in general with the original description except that the sclerotia range from about 190μ to 310μ in diameter. These measurements agree in general with those given by Tisdale (8) and Tullis (9) for the sclerotia of the fungus in the United States.

The sclerotia of *Helminthosporium sigmoideum irregulare* differ from those of *Sclerotium oryzae* not only in being irregular but also in being distinctly smaller. They measure only 90μ to 110μ by 268μ to 342μ . Morphological differences in the sclerotia of *Leptosphaeria salvinii* and *H. sigmoideum irregulare* are shown in figures 2, A, B, and 3, A, B.

Sclerotia of *Leptosphaeria salvinii* and *Helminthosporium sigmoideum irregulare* in culture are shown in figure 4, A, B. The most striking difference between them is that the sclerotia of *H. sigmoideum irregulare* usually are embedded in the medium and are formed as irregular masses on the radiating strands of the hyphae, whereas sclerotia of *L. salvinii* are spherical or nearly so and are formed individually and as abundantly by the aerial as by the submerged hyphae but not in radiating rows.

The conidia of *Helminthosporium sigmoideum irregulare* are similar in appearance and size to those of *Leptosphaeria salvinii*, as is shown in figures 4, C, D. Some conidia of the former tend to produce germ tubes from the apex while still attached to the conidiophore, as is shown in figure 4, E. In some cases this occurs before cross walls have formed in the spore. Such germ-tube formation has never been observed in the conidia of *L. salvinii* while the spores are still attached to the conidiophores. When grown in artificial culture, frequently one or more septa in the spores of *H. sigmoideum irregulare* are lacking, so that the spores are not so uniformly three-septate as are the conidia of *L. salvinii*. A count was made of the septation of 53 spores of *H. sigmoideum irregulare* in a culture from Arkansas. The results were as follows: Spores without septa, 25; spores with 1 septum, 2; spores with 2 septa, 10; spores typically three septate, 16. Of this number, 50 had germ tubes and 3 had none. Cultures from single conidia of *L. salvinii* with few exceptions have produced only the conidial stage of the fungus, even after

³ Received from Dr. K. Nakata, Kyushu Imperial University, Fukuoka, Japan.

⁴ Received from D. T. G. Fajardo, Philippine Bureau of Science, Manila, P. I.

⁵ RABENHORST, L. FUNGI EUROPAE EXSICCATI. Ed. nova, ser. 2, Cent. 5, no. 2460. Dresdae. 1876.



Rabenhorst, Fungi europaei
2460. Sclerotium Oryzae Catt. Rendic. del
R. Istituto Lombardo di Scienze e Lettere, Ser. II. V.
IX. fasc. XX. Arch. triennale del Laboratorio di Botanica
Critt. di Pavia. Vol. II. con una tavola.

A Oryzeta Ticin. et Novar. 1876.

leg. A. Cattaneo.



FIGURE 1.—A, Reproduction of cotype material of sclerotia of *Leptosphaeria salvinii*, $\times 1$; B, enlargement of portion of lower culm in A, to show spherical form of the sclerotia. $\times 30$.

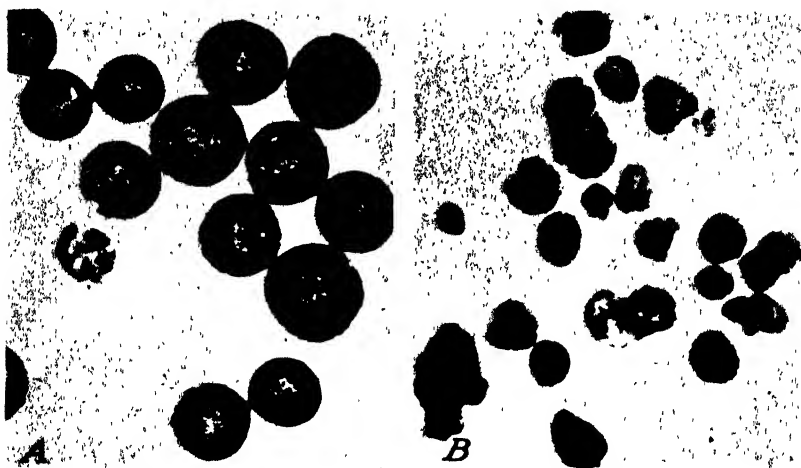


FIGURE 2.—Sclerotia of *Leptosphaeria satrini* (A) and *Helminthosporium sigmoideum* (B) from culms of Blue Rose rice $\times 40$.



Figure 3.—Stem-rot-diseased culms of Supreme Blue Rose rice: A, Sclerotia of *Leptosphaeria satrini*, $\times 3$; B, sclerotia of *Helminthosporium sigmoideum irregulare*. $\times 3$.

numerous successive transfers, whereas cultures from single conidia of *H. sigmoideum irregulare* consistently have produced sclerotia at first and conidia later.

Numerous attempts have been made to determine whether typical spherical sclerotia of *Leptosphaeria salvinii* might be produced by transfers of sclerotia or conidia of *Helminthosporium sigmoideum irregulare* or from isolations of the fungus from diseased plants, but the attempts have given negative results. Other experiments were made in which *H. sigmoideum irregulare* was grown on various media, including sterilized rice straw and rice-straw ground and incorporated in agar of various kinds, in attempts to produce the sclerotia typical

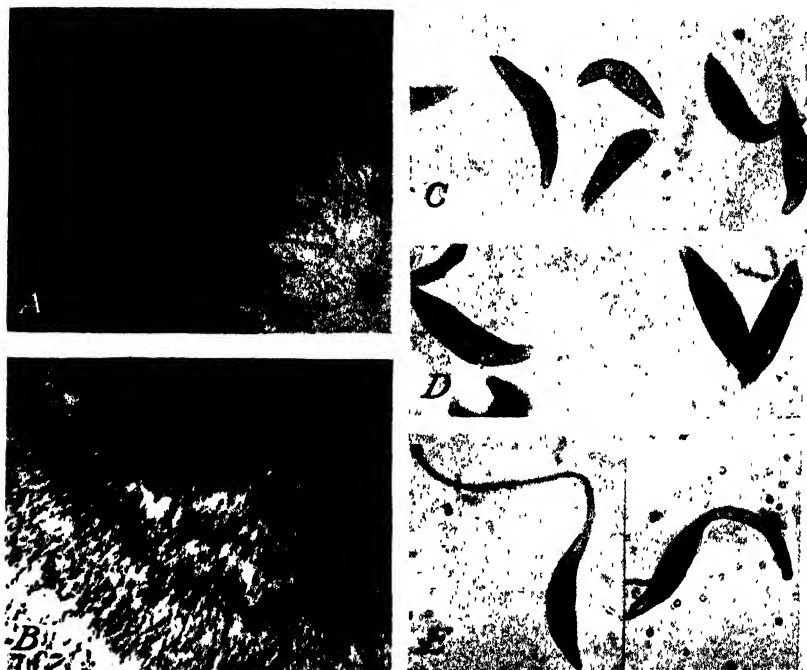


FIGURE 4. -A, Portion of a colony of *Leptosphaeria salvinii* growing on corn-meal agar. $\times 15$; B, portion of a colony of *Helminthosporium sigmoideum irregulare* growing on corn-meal agar. $\times 15$; C, conidia of *H. sigmoideum irregulare*. $\times 320$; D, conidia of *L. salvinii*. $\times 400$; E, conidia of *H. sigmoideum irregulare* with germin tubes. $\times 320$.

of *L. salvinii*. In all cases, regardless of the appearance of the sclerotia on these media, no sclerotia typical of *L. salvinii* were produced when transferred back to corn-meal agar. *H. sigmoideum irregulare* produced conidia readily on an agar composed of 1 percent pectin and 4 percent agar.

In a previous paper (10) the writers described the production of conidia by sclerotia of *Leptosphaeria salvinii* floating on water. *Helminthosporium sigmoideum irregulare* also produces conidia under these conditions. Sclerotia from Louisiana and from plants infected with the culture received from Saga, Japan, were used. The first conidia were produced in 7 and 20 days, respectively, and conidial formation continued for 5 months, at which time the experiment was discontinued. During this period as many as 40 conidia were pro-

duced by some of the sclerotia. Individual sclerotia of *L. salvinii* under similar conditions in the same period have produced as many as 75 conidia.

Since this manuscript was prepared for publication the attention of the writers has been called to a book by Nakata (5) on crop diseases,⁶ published in Tokyo, September 15, 1934. In the discussion of these diseases, Nakata describes as *Helminthosporium sigmoideum* Cav. the fungus with irregular sclerotia, which the writers have described (4) as *H. sigmoideum irregulare*; and he describes as *H. sigmoideum* var. *microsphaeroides* Nakata the fungus with spherical sclerotia, which has been shown to be *Leptosphaeria salvinii* Catt.

As previously shown (10), and again pointed out in the present paper, the fungus with spherical sclerotia, *Leptosphaeria salvinii* Catt., has *Sclerotium oryzae* Catt. as its sclerotial stage and *Helminthosporium sigmoideum* Cav. as its conidial stage. On the basis of the descriptions and measurements of sclerotia and conidia given by Nakata (5, p. 24), it is evident that his *H. sigmoideum microsphaeroides* is identical with the sclerotial and conidial stages of *L. salvinii*. Furthermore, the writers have secured a culture from Institut voor Schimmelcultures at Baarn, Netherlands, which had been supplied by Nakata. This culture was labeled *Sclerotium oryzae*, and in all essentials is identical with cultures of *L. salvinii* from the United States, India, and the Philippine Islands. It is evident, therefore, that the name of this fungus should be *L. salvinii* Catt.

From the descriptions and measurements of sclerotia and conidia given by Nakata (5, p. 22) for the fungus with irregular sclerotia, it is clear that the fungus which he has included under the name *Helminthosporium sigmoideum* Cav. in reality is the fungus which the writers have described as *H. sigmoideum* var. *irregulare* (4). Cultures of "*H. sigmoideum*" supplied the writers by Nakata are identical with cultures of the fungus which they have described as *H. sigmoideum* var. *irregulare*. Even the characteristic germ tubes of the conidia are identical. The formation of germ tubes by the conidia while still attached to the conidiophores, is a characteristic of the conidia of *H. sigmoideum* var. *irregulare* not found in those of *Leptosphaeria salvinii*. These germ tubes have been observed by the writers on numerous occasions on conidia from the cultures received from Nakata as "*H. sigmoideum*." It is evident, therefore, that the fungus which Nakata has called *H. sigmoideum* Cav., is identical with that which the writers have described as *H. sigmoideum* var. *irregulare* and that the true *H. sigmoideum* Cav. is the conidial stage of *Leptosphaeria salvinii* Catt.

According to the above interpretation, the synonymy for these two fungi is as follows:

- (1) *Leptosphaeria salvinii* Catt. Synonyms for conidial and sclerotial stages:
Helminthosporium sigmoideum Cav.
Helminthosporium sigmoideum Cav. var. *microsphaeroides* Nakata
Sclerotium oryzae Catt.
- (2) *Helminthosporium sigmoideum* Cav. var. *irregulare* Cralley and Tullis.
Synonym:
Helminthosporium sigmoideum Nakata, not Cav.

⁶ Saburo Katsura has kindly translated portions of the text dealing with the sclerotial diseases of rice.

PATHOGENICITY OF *HELMINTHOSPORIUM SIGMOIDEUM* IRREGULARE

Inoculation experiments were conducted to test the pathogenicity of *Helminthosporium sigmoideum irregulare* and also to test its ability to produce conidia on the host.

Inoculation tests were made in the laboratory and in the greenhouse. The first inoculations were made by placing bits of mycelium on agar adjacent to single sterile seedlings of Supreme Blue Rose rice growing on corn-meal agar in test tubes. The results of these inoculations are shown in table 1.

Ninety-day-old plants of Supreme Blue Rose growing in the greenhouse in stoneware jars were inoculated in duplicate with 8 cultures. Inoculations with 7 cultures were made by inserting bits of mycelium on agar under the outer leaf sheaths. Inoculation with culture 8 was made by scattering sclerotia on the surface of the water. The source of the cultures and the results of these inoculations are shown in table 2.

TABLE 1.—Results of inoculation of rice seedlings growing under aseptic conditions on corn-meal agar in test tubes with 8 cultures of *Helminthosporium sigmoideum irregulare*

Source of culture	Plants inoculated ¹	Plants infected	Source of culture	Plants inoculated ¹	Plants infected
	Number	Percent		Number	Percent
Japan:			United States:		
Kumamoto	4	100	Louisiana (Crowley)	4	100
Okayama	4	100	Arkansas	10	100
Hiroshima	4	100	Control, 12 plants not inoculated		0
Saga	4	100			

¹ The conidial stage was not observed on any of the inoculated dead plants.

TABLE 2.—Results of inoculation of Supreme Blue Rose rice plants in the greenhouse with sclerotia of the various cultures of *Helminthosporium sigmoideum irregulare*, 1932-33

Culture No.	Source	Plants inoculated	Plants infected		Culture No.	Source	Plants inoculated	Plants infected	
		Number	Number	Percent			Number	Number	Percent
1	Japan: Kumamoto ..	23	3	13.0	6	Japan: Fukuoka	25	0	.0
2	Okayama	28	1	3.6	7	Okayama	71	12	16.9
3	Hiroshima	67	19	28.4	8	Arkansas	232	99	42.7
4	Saga	61	15	24.6		Average			34.0
5	Louisiana	212	112	52.8		Control, 61 plants not inoculated ..		0	.0

¹ Typical conidia of *H. sigmoideum irregulare* were produced on these infected plants.

Nearly 35 percent of the total number of plants inoculated became infected. As compared with the rather high percentage of infection obtained from the cultures from Arkansas and Louisiana—42.7 and 52.8, respectively—the percentages of infection from those obtained from Japan were rather low and irregular, ranging from 0 to 28.4. However, the number of plants inoculated with these cultures was not

large, and the cultures may possibly have been rather old. The results demonstrate, however, that *Helminthosporium sigmoideum irregulare* is able to infect healthy plants and to produce in and on them sclerotia and conidia similar to the forms used in the inoculations. The fungus was reisolated from the diseased plants.

SUMMARY

A description of *Helminthosporium sigmoideum* var. *irregulare* is presented.

A comparison of the morphology of *Leptosphaeria salvinii* and *Helminthosporium sigmoideum* var. *irregulare* is given and the pathogenicity of the latter on rice is shown.

LITERATURE CITED

- (1) CATTANEO, A.
1876. SULLO SCLEROTIUM ORYZAE, NUOVO PARASSITA VEGETALE CHE HA DEVASTATE NEL CORRENTE ANNO MOLTE RISAJE DI LOMBARDIA E NEL NOVARESE. R. Ist. Lombardo Sci. e Let. Rend. (2) 9: 801-807. [Also published in Arch. Lab. Bot. Crittogamica R. Univ. Pavia 2-3: [75]-83, with plate VII added. 1879.]
- (2) ———
1879. CONTRIBUTO ALLO STUDIO DEI MICETI CHE NASCONO SULLE PIANTICELLE DI RISO. Arch. Lab. Bot. Crittogamica R. Univ. Pavia 2-3: [115]-128, illus.
- (3) CAVARA, F.
1889. MATÉRIAUX DE MYCOLOGIE LOMBARDE. Rev. Mycol. 11: 185, illus.
- (4) CRALLEY, E. M., and TULLIS, E. C.
1934. RICE DISEASE INVESTIGATIONS—STEM ROT. Ark. Agr. Expt. Sta. Bull. 312 (Ann. Rept. 46): 52-53.
- (5) NAKATA, K.
1934. SAKUMOTSU BYOGAI ZUHEN [ILLUSTRATED CROP DISEASES]. Tokyo.
- (6) PARK, M. and BERTUS, L. A.
1934. SCLEROTIAL DISEASES OF RICE IN CEYLON. IV SCLEROTIUM ORYZAE A STRAIN. Ann. Roy. Bot. Gard. Peradeniya 12 (pt. 1).
- (7) SAKURAI, M.
1917. ON SCLEROTIUM DISEASES OF RICE PLANT. Ehime Agr. Expt. Sta. Pub. 1, 51 pp., illus. [In Japanese. English abstract in Bot. Abs. 10: 195, 1922.]
- (8) TISDALE, W. H.
1921. TWO SCLEROTIUM DISEASES OF RICE. Jour. Agr. Research 21: 649-658, illus.
- (9) TULLIS, E. C.
1933. LEPTOSPHAERIA SALVINII, THE ASCIGEROUS STAGE OF HELMINTHOSPORIUM SIGMOIDEUM and SCLEROTIUM ORYZAE. Jour. Agr. Research 47: 675-687, illus.
- (10) ——— and CRALLEY, E. M.
1933. LABORATORY AND FIELD STUDIES ON THE DEVELOPMENT AND CONTROL OF STEM ROT OF RICE. Ark. Agr. Expt. Sta. Bull. 295, 23 pp., illus.

COMPARATIVE TOXICITY OF ANABASINE AND NICOTINE SULPHATES TO INSECTS¹

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HISTORICAL REVIEW

Within the last few years the Government of the Union of Soviet Socialistic Republics has placed anabesine sulphate on the American market as a new contact poison selling at a price considerably lower than nicotine sulphate. Anabesine is an alkaloid present in the stems and leaves of *Anabasis aphylla* L., a member of the Chenopodiaceae. It is somewhat similar to nicotine in its structural formula and has the same empirical formula ($C_{10}H_{14}N_2$). Anabesine is considered an isomer (7)² of nicotine. Like nicotine, it can be readily extracted from the plant and converted into a sulphate.

Aphylla is only one of a large number of species belonging to the genus *Anabasis*. It is a perennial weed growing (10) in northern Africa, Russia, Armenia, and in the neighboring countries. Attempts are at present being made to cultivate it on the North American continent. The amount of alkaloid varies from a fraction of 1 percent in old twigs and thick leaves to more than 2 percent in young succulent twigs and young growing leaves. The commercial product anabesine sulphate contains approximately 40 percent of total alkaloids, of which about 70 percent is anabesine (1, 7), the remainder being lupinine, other higher alkaloids, and miscellaneous plant material.

It may be of interest that not long before Orechhoff and Menschikoff (8) had isolated anabesine from the plant, Smith (9) synthesized, in 1930, the alkaloid β -pyridyl- α -piperidine, which he called "neonicotine" and which he later (11) found to be chemically the same as the natural anabesine. The only difference between the two is that neonicotine is optically inactive while anabesine is levorotatory. Smith, Richardson, and Shepard (12) found neonicotine as toxic to *Aphis rumicis* L. as nicotine.

Garman (2, 3, 4) reports that both anabesine and anabesine sulphate are more toxic to aphids than are nicotine and nicotine sulphate. In his experiments, dilutions of 1 pint of either anabesine sulphate or nicotine sulphate to 100 gallons of water produced equally high control of the white apple leafhopper. On the other hand, Campbell, Sullivan, and Smith (1) found anabesine less toxic than nicotine to culicine mosquito larvae.

¹ Received for publication May 17, 1935; issued October 1935. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Entomology.

² Reference is made by number (italic) to Literature Cited, p. 354.

EXPERIMENTAL WORK

In May 1931 the senior writer tested the toxicity to honeybees of anabasine sulphate received from the Amtorg Trading Corporation. Applied as a contact spray, concentrations of 0.2 and 0.1 percent killed 100 percent of the bees within 24 hours. When the properties of anabasine, as an internal poison, were tested by allowing the bees to feed on honey containing 0.2 percent of anabasine sulphate, only about 10 percent died within the same period. On the other hand, in a previous publication (5) the senior writer reported high percentages of kill of bees fed on honey containing 1 part of nicotine, in the form of nicotine oleate, to 3,200 parts of honey mixture. These preliminary results suggested that anabasine is much more effective as a contact poison than as a stomach poison.

Experiments with anabasine sulphate were resumed during the summer of 1934. Since nicotine is largely used in agricultural sprays to control aphids, the major part of this investigation was devoted to comparing the aphicidal properties of the two insecticides. The samples used in these tests contained 40.5 percent and 40 percent total alkaloids for nicotine sulphate and anabasine sulphate, respectively, as stated in the chemical analysis submitted by the manufacturers.

The tests with various dilutions of anabasine sulphate and nicotine sulphate were conducted on several species of aphids, on silk moth larvae, and on grasshoppers. The toxicity to insects was determined by methods previously described (6); 90 percent kill or higher was considered efficient control.

TESTS ON APHIDS

The following species of aphids were used in these tests: *Aphis pomi* De G., on apple; *Aphis rumicis*, Lin. on nasturtium; *Macrosiphum rosae* Lin. on roses; *Macrosiphoniella sanborni* Gill and *Rhopalosiphum rufomaculata* Wils., on chrysanthemum. In order to spread and wet efficiently, each spray solution had added to it 0.2 percent of coconut-oil soap. Several series of tests were run for each dilution. The average results are reported in the tables.

LABORATORY TESTS

Check tests with the wetting agent alone have shown that 0.2 percent of soap killed approximately 14 percent of the green apple aphids (*Aphis pomi*), 21 percent of the nasturtium aphids (*A. rumicis*), and 13 percent of *Macrosiphum rosae*. The concentrations of the spray mixtures ranged from 1 pint to one twenty-fourth of a pint per 100 gallons. The high dilutions, although not practical, were necessary in order to evaluate the differences between the toxicity of the two alkaloids. The results from tests on the aphids are set forth in table 1. A comparison of the results with apple and nasturtium aphids reveals no distinct differences in toxicity of the two insecticides in concentrations of one-half or one-third of a pint per 100 gallons, the percentage of kill being 90 or better in each case. At concentrations of one-sixth and one-twelfth of a pint the results were

decidedly higher, on both species of aphids, with anabesine sulphate than with nicotine sulphate. The rose aphids were more resistant to both anabesine and nicotine, 1 pint of anabesine sulphate being required per 100 gallons to produce about a 90 percent kill; the percentages of dead rose aphids were consistently higher with anabesine sulphate than with nicotine sulphate.

TABLE 1.—*Toxicity tests with anabesine and with nicotine sulphates on green-apple aphids (Aphis pomi), nasturtium aphids (A. rumicis), and rose aphids (Macrosiphum rosae)*

Insecticide in 100 gallons of spray ¹ (pints)	Green-apple aphids		Nasturtium aphids		Rose aphids	
	Total insects	Dead after 24 hours	Total insects	Dead after 24 hours	Total insects	Dead after 24 hours
Anabesine sulphate:	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>
1.....					321	90.8
$\frac{1}{2}$	888	94.8			436	78.4
$\frac{1}{4}$	1,342	97.6	511	95.8	334	66.0
$\frac{1}{8}$	618	93.4	322	90.9	608	60.0
$\frac{1}{12}$	1,098	66.4	447	78.0		
$\frac{1}{24}$	745	31.0	370	53.7		
Nicotine sulphate:						
1.....					372	87.6
$\frac{1}{2}$	1,200	97.3			336	59.0
$\frac{1}{4}$	1,736	90.2	272	92.6	471	52.4
$\frac{1}{8}$	435	82.3	272	86.0	383	48.5
$\frac{1}{12}$	1,982	58.9	788	66.7		
$\frac{1}{24}$	686	38.1	273	48.7		
Check, 0.2 percent of soap: 0.....	1,338	14.7	716	21.4	301	13.0

¹ A concentration of 1 pint of insecticide per 100 gallons of spray represents an approximate dilution of the alkaloids of 1 to 2,000.

GREENHOUSE TESTS

Several beds of chrysanthemum plants, in a commercial greenhouse, were divided into two parts and sprayed with the two insecticides on August 2, 1934. In each case only one concentration, namely, one-third of a pint of insecticide per 100 gallons of spray solution, was used. The plants were infested with two different species of aphids, the black (*Macrosiphoniella sanborni*) and the green (*Rhopalosiphum rufomaculata*). After approximately 24 hours, gross observations showed a practically complete kill of the black aphids and an incomplete kill of the green aphids with each of the spray mixtures. In order more closely to verify these differences, several plants were removed from each plot, and counts of live and dead aphids were made. The results, set forth in table 2, show 100-percent control of black aphids with both spray mixtures. The control of green aphids disclosed striking differences in favor of the anabesine sulphate, the actual percentages killed being 87.8 for anabesine sulphate and 32.1 for nicotine sulphate. The experiments were repeated on August 8 on different chrysanthemum plants in the same greenhouse. Only the green aphids were counted. The results (table 2) again show high kill with anabesine sulphate and low kill with nicotine sulphate. It appears, therefore, from these results that anabesine possesses higher toxicity to this species of aphids than does nicotine.

TABLE 2.—*Toxicity tests with anabesine and with nicotine sulphates*¹ on two species of aphids, *Macrosiphoniella sanborni* and *Rhopalosiphum rufomaculata*, infesting *chrysanthemum* plants

Insecticide tested	Date of spraying	Black aphids, <i>Macrosiphoniella sanborni</i>		Green aphids, <i>Rhopalosiphum rufomaculata</i>	
		Total insects on 4 plants	Dead after 24 hours	Total insects on 4 plants	Dead after 24 hours
		<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>
Anabesine sulphate.....	Aug. 2, 1934	297	100	296	87.8
Nicotine sulphate.....	do.....	173	100	205	32.1
Anabesine sulphate.....	Aug. 8, 1934	-----	-----	31	87.1
Nicotine sulphate.....	do.....	-----	-----	329	28.8

¹ One-third pint of insecticide per 100 gallons of spray.

TESTS ON CHEWING INSECTS

LABORATORY TESTS ON SILK MOTH LARVAE

The silk moth larva (*Bombyx mori* L.) has proved to be a satisfactory insect-indicator for testing stomach poisons in this laboratory. A comparison of the toxicity of anabesine and nicotine sulphates to healthy silk moth caterpillars was, therefore, made. Because of the scarcity of the insects at the time of testing, only 10 insects, all in the fourth instar, were used for each single test. In order to eliminate volatility of the alkaloids, 0.1 percent of a neutral wetting agent³ was added to each spray mixture, instead of soap to produce efficient spreading.

The results, presented in table 3 show that concentrations of 1 quart of nicotine sulphate to 100 gallons of water gave a 100-percent kill in 2 days, whereas anabesine sulphate gave a 30-percent kill in 3 days. In concentrations of 1 pint to 100 gallons, nicotine sulphate gave about 95-percent kill and anabesine sulphate only 15-percent kill in 3 days.

TABLE 3.—*Toxicity tests with anabesine and with nicotine sulphates on silk-moth larvae, using 10 larvae per test*

Insecticide tested	Insecticide per 100 gallons of spray ¹	Dead—		
		After 1 day	After 2 days	After 3 days
	<i>Pints</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Nicotine sulphate.....	2	90	100	-----
Do.....	1	40	90	² 100
Do.....	1	30	70	² 90
Anabesine sulphate.....	2	0	10	² 30
Do.....	1	0	0	² 10
Do.....	1	0	10	² 20
Check (0.1 percent of arescap).....	0	0	0	0
Do.....	0	0	0	0

¹ See note, table 1, for approximate dilution of the alkaloids.

² Average, 95.

³ Average, 15.

⁴ Arescap obtained from Monsanto Chemical Co., St. Louis, Mo.

LABORATORY TESTS ON GRASSHOPPERS

Adults of a short-horned grasshopper (*Melanoplus femur-rubrum*, De G.), commonly called "red-legged locust", collected from a meadow near the college campus, were transferred to cages and fed on young potted tomato plants, sprayed 1 hour previously, with various concentrations of nicotine sulphate and anabasine sulphate. Twenty insects were used for each test. Each spray solution contained 0.1 percent of the neutral wetting agent (Arescap). The tomato plant is evidently very palatable to this species of grasshopper, since the insects rapidly devoured the leaves on the check plants, sprayed only with 0.1 percent of Arescap, so that the plants had to be renewed once during the 3 days of testing. The feeding on the plants sprayed with the two insecticides, however, was rapidly retarded after the first day especially on those sprayed with nicotine sulphate, and in neither case was the renewal of plants necessary.

The results, presented in table 4, show that nicotine sulphate is decidedly more toxic to this chewing insect than is anabasine sulphate. At concentrations of 1 pint and 1 quart per 100 gallons of spray, nicotine sulphate produced 80- and 90-percent kill, respectively, as compared with 40- and 60-percent mortality obtained with the same concentrations of anabasine sulphate.

TABLE 4.—Toxicity tests with anabasine and with nicotine sulphates on grasshoppers, in which 20 insects per test were used

Insecticide tested	Insecticide per 100 gallons of spray	Dead after 3 days
	<i>Pints</i>	<i>Percent</i>
Nicotine sulphate.....	1	80
Do.....	2	90
Anabasine sulphate.....	1	40
Do.....	2	60
Check, 0.1 percent of Arescap.....	0	15

SUMMARY AND CONCLUSIONS

Laboratory and greenhouse tests were conducted with anabasine sulphate and nicotine sulphate on several species of aphids, silk moth larvae, grasshoppers, and honeybees. The results definitely show that:

Anabasine sulphate equals or excels nicotine sulphate in toxicity to *Aphis rumicis*, *A. pomi*, and *Macrosiphoniella sanborni* and is decidedly more toxic to *Rhopalosiphum rufomaculata* and *Macrosiphum rosae*.

Anabasine sulphate possesses very little toxicity as a stomach poison against silk moth larvae, while nicotine sulphate proved highly toxic to this insect.

Anabasine sulphate was decidedly less toxic to grasshoppers, applied as a stomach poison, than was nicotine sulphate.

LITERATURE CITED

- (1) CAMPBELL, F. L., SULLIVAN, W. N., and SMITH, C. R.
1933. THE RELATIVE TOXICITY OF NICOTINE, ANABASINE, METHYL ANABASINE,
AND LUPININE FOR CULICINE MOSQUITO LARVAE. *Jour. Econ. Ent.*
26: 500-509. illus.
- (2) GARMAN, P.
1933. NOTES OF THE COMPARATIVE TOXICITY OF ANABASINE SULFATE AND
NICOTINE SULFATE FOR APHIS AND LEAFHOPPERS. *Conn. State Agr.*
Expt. Sta. Bull. 349: 433-434.
- (3) ———
1934. STUDY OF APHICIDES. *Conn. State Agr. Expt. Sta. Bull.* 360: 458-
461. illus.
- (4) ——— and TOWNSEND, J. F.
1934. CONTROL OF THE WHITE APPLE LEAFHOPPER, 1933. *Conn. State.*
Agr. Expt. Sta. Bull. 360: 449-451.
- (5) GINSBURG, J. M.
1928. INSECTICIDE INVESTIGATIONS. *N. J. Agr. Expt. Sta. Rept.* (1927-28)
49: 158-163.
- (6) ——— SCHMITT, J. B., and GRANETT, P.
1934. DERRIS INSECTICIDES: I. TOXICITY OF VARIOUS EXTRACTS OF DERRIS
ROOT TO SUCKING AND CHEWING INSECTS. *N. J. Agr. Expt. Sta.*
Bull. 576: [3]-16.
- (7) NELSON, O. A.
1934. SOME PHYSICAL CONSTANTS OF ANABASINE. *Jour. Amer. Chem. Soc.*
56: 1989-1990.
- (8) ORECHOFF, A., and MENSCHIKOFF, F.
1931. ÜBER DIE ALKALOIDE VON ANABASIS APHYLLA L. (I. MITTEIL.) *Ber.*
Deut. Chem. Gesell. 64: 266-274.
- (9) SMITH, C. R.
1931. NEONICOTINE AND ISOMERIC PYRIDYLPIPERIDINES. *Jour. Amer. Chem.*
Soc. 53: 277-283.
- (10) ———
1931. NEONICOTINE RECENTLY FOUND AS AN ALKALOID IN ANABASIS
APHYLLA L. (Sci. Note) *Jour. Econ. Ent.* 24: 1108.
- (11) ———
1932. IDENTITY OF NEONICOTINE AND THE ALKALOID ANABASINE. *Jour.*
Amer. Chem. Soc. 54: 397-399.
- (12) ——— RICHARDSON, C. H., and SHEPARD, H. H.
1930. NEONICOTINE AND CERTAIN OTHER DERIVATIVES OF THE DIPYRIDYLS AS
INSECTICIDES. *Jour. Econ. Ent.* 23: 863-867.

THE TOXICITY OF OPTICALLY ACTIVE AND INACTIVE DIHYDRODEGUELINS¹

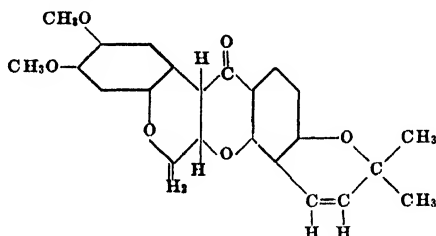
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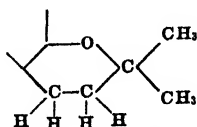
INTRODUCTION

During a study of toxic constituents of derris root other than rotenone, Haller and LaForge prepared two dihydrodeguelins, one optically active and the other optically inactive (?).² Since the physiological action of a compound may depend on its configuration as well as on its chemical constitution, it was considered of value to study these two derivatives toxicologically.

Although Haller and LaForge found the "deguelin concentrate"—the amorphous residue left after separation of the rotenone and toxocarol present in petroleum ether extract of derris root—to be strongly levorotatory, they were unable to obtain crystalline active deguelin from it. Thus, the inactive modification was the only form available for comparative purposes. This compound was prepared by these workers by treatment of deguelin concentrate with alkali. However, the sample of inactive deguelin (melting point 171° C.) used in the following study was prepared by Clark (1). Its constitution (2) is expressed by the formula:



By catalytic hydrogenation of deguelin concentrate Haller and LaForge (?) obtained levodihydrodeguelin (melting point 155°–156° C.). After separating the portion that crystallized out, they isolated as the inactive form (melting point 171°) the dihydrodeguelin remaining in the mother liquor by alkali treatment and subsequent crystallization. The structure of these dihydro derivatives differs from that of deguelin only by saturation with hydrogen at the double bond in the chroman system, thus:



¹ Received for publication June 27, 1935; issued October 1935.

² Reference is made by number (italic) to Literature Cited, p. 360.

It is the presence of this system instead of the benzofuran system which differentiates these compounds from their isomers, rotenone and dihydrorotenone (8).

EXPERIMENTAL PROCEDURE

The method used in studying the toxicity of these compounds has been described previously (3). Goldfish of the same lot, weighing from 2.7 to 3.7 g each, were used in all the tests, and a constant temperature (27° C.) was maintained. For comparative purposes tests were also made with rotenone. In addition, because a definite relationship has been established between certain dihydro derivatives of rotenone and their parent compounds, it was thought of interest to make tests with deguelin.

RESULTS

The toxicological data are given in table 1. The survival-time curves and the velocity-of-fatality curves, which are plotted from these data, are given in figures 1 and 2.

TABLE 1.—*Toxicity of rotenone, deguelin, and active and inactive dihydrodeguelins to goldfish at 27.0° ± 0.2° C.*

Compound	Concentration	Fishes used	Mean length of fishes	Mean weight of fishes ¹	Mean survival time	Mean ^{1,000} survival time
	<i>Milligram per liter</i>	<i>Number</i>	<i>Milli-meters</i>	<i>Grams</i>	<i>Minutes</i>	
Rotenone.....	0.900	14	46	2.9	127	8.06
	.600	16	48	3.3	131	7.98
	.500	13	48	3.3	144	7.14
	.400	12	48	3.3	149	6.86
	.300	12	49	3.4	154	6.93
	.200	13	46	2.9	192	5.63
	.150	13	48	3.3	229	4.78
	.120	11	46	2.9	250	4.20
	.100	27	47	3.1	280	3.91
	.075	13	48	3.3	377	2.76
	.050	12	48	3.3	540	1.95
	.030	12	50	3.7	780	1.27
	.700	9	48	3.3	152	6.91
	.400	12	47	3.1	168	6.03
	.300	11	49	3.5	181	5.73
Deguelin.....	.250	34	48	3.3	204	5.25
	.200	46	48	3.3	238	4.61
	.150	34	47	3.2	269	3.75
	.120	11	48	3.3	380	3.17
	.100	14	48	3.3	455	2.36
	.900	10	47	3.0	126	8.13
	.500	12	45	2.7	132	7.72
	.400	15	45	2.7	147	6.90
	.300	11	47	3.0	154	6.84
	.250	16	47	3.1	159	6.46
Active dihydrodeguelin.....	.200	27	48	3.3	199	5.32
	.150	13	47	3.1	242	4.35
	.120	11	46	2.9	262	4.21
	.100	28	49	3.5	352	3.10
	.075	11	49	3.5	474	2.24
	.030	9	(²)	(²)	(³)	1.55
	.900	10	47	3.1	159	6.36
Inactive dihydrodeguelin.....	.600	13	47	3.1	181	5.68
	.500	11	48	3.3	213	5.15
	.400	13	49	3.4	224	4.94
	.300	12	47	3.0	269	4.12
	.240	12	49	3.5	295	3.71
	.200	13	47	3.1	392	2.98
	.150	9	48	3.3	496	2.24
	.100	12	46	3.0	930	1.17

¹ Estimated from length, which measurement excludes the tail.

² Fishes not measured but of same approximate size.

³ 5 fishes survived 17 hours; 4 fishes were apparently unaffected after 48 hours. These figures are only approximate, since a large number of fishes would be required to give an accurate mean value. The reciprocal of the survival time of a fish surviving the test is taken as zero, since the reciprocal of any survival time longer than the test would be negligibly small.

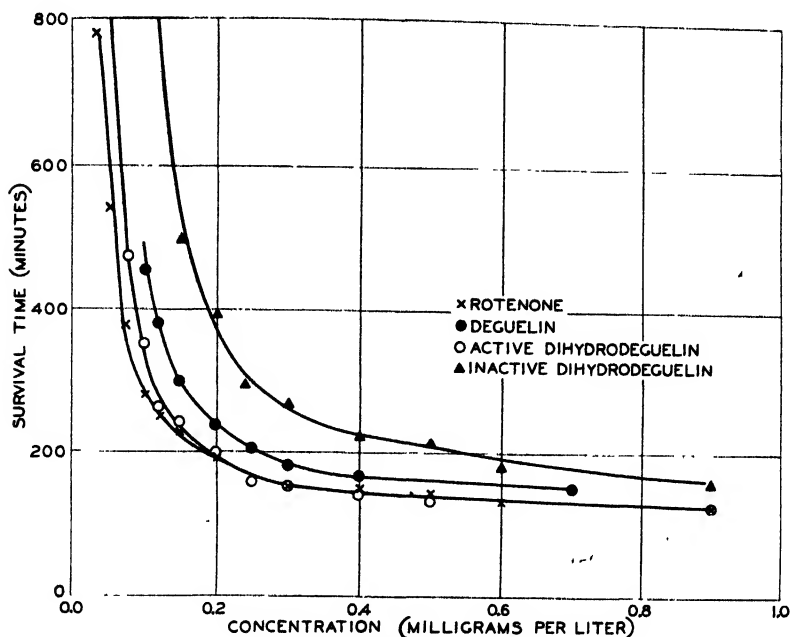


FIGURE 1.—Survival-time curves.

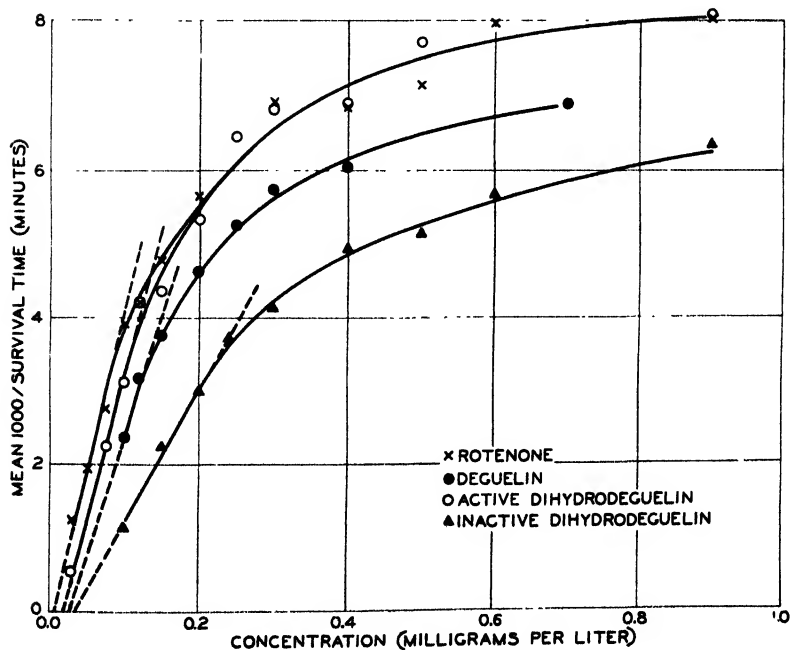


FIGURE 2.—Velocity-of-fatality curves. Straight lines indicate approximation of that portion of the curve corresponding to greatest rate of increase of velocity of fatality with increase in concentration.

DISCUSSION

Comparative data obtained from these curves are given in table 2. In figure 2 the straight line, which is an approximation of that portion of the curve corresponding to the greatest rate of increase in the velocity of fatality with increase in concentration, is prolonged to cut the X-axis at a point designated " c_0 "; the slope of this line is designated " $\tan \theta$ ", and its measurement is expressed in the dimensions of both coordinates. In this way values are obtained, respectively, for the theoretical threshold of toxicity, or the concentration below which the substance does not cause death, and for the maximum rate of increase of the velocity of fatality with increase in concentration. In a previous study (5) of nine substances with relatively low thresholds a point on the survival-time curve called the "minimum ct product", $(ct)_m$, was found to correspond very closely to the upper end of this straight line, and this fact has been employed in locating the individual lines. Beyond the arching portion of either the survival-time curve or the velocity-of-fatality curve in the direction of higher concentrations it approaches a region of constant velocity; that is, the curve approaches the horizontal. To obtain values for comparative purposes, the region in which the survival times corresponding to concentrations, one of which is double the other, do not differ by more than 5 percent is chosen arbitrarily as the minimum survival time and designated " t_0 ." The values given for t_0 in the table are approximated from the respective survival-time curves.

TABLE 2.—Relative toxicity at 27° C. of rotenone, deguelin, and optically active and inactive dihydrodeguelins

Compound	c_0	$\tan \theta$	$\sqrt{\frac{\tan \theta}{c_0}}$	t_0	c_m	t_m	$(ct)_m$	Relative toxicity according to $(ct)_m$
	Milligram per liter	Liter per milligram per minute		Minutes	Milligram per liter	Minutes	Milligram minutes per liter	
Rotenone.....	0.01	0.044	2.1	120	0.085	320	27.2	1.00
Deguelin.....	.03	.033	1.1	130	.135	329	44.4	.614
Active dihydrodeguelin.....	.02	.040	1.4	120	.110	302	33.2	.819
Inactive dihydrodeguelin.....	.035	.018	.72	140	.220	333	73.3	.371

The minimum product of concentration and time, $(ct)_m$, was suggested in a previous paper (5) as a suitable criterion for the comparison of toxicity, since it gives a relative value at the point of greatest efficiency of the toxic substance with respect to concentration and time. The value is determined from the survival-time curve; its probable error is less than 7 percent of the mean survival time. Its coordinates, c_m and t_m , are given in order that the point can be located on the curve.

It is apparent from columns 3 and 9 of table 2 that, compared according to either of the methods in which the toxicity of a substance is considered only at its highest efficiency with respect to concentration and time, these compounds have the following descending order of toxicity: Rotenone, active dihydrodeguelin, deguelin, and inactive dihydrodeguelin. Compared according to the values ob-

tained for the respective thresholds of toxicity, c_0 , this order still obtains. It must be emphasized, however, that these values are obtained under theoretical conditions and may have no real significance beyond showing the order of toxicity at very great dilutions. Also they are approximate only, since many more observations would be necessary to distinguish between the closer values with accuracy. Similarly, the values given for the minimum survival time, t_0 , are only approximate and for the region of the curves as indicated above. There is no evidence that these values would differ if the survival times were compared at concentrations above those given at the same relative portions of their curves; the indications are that they would be close together. An extrapolative procedure such as was used to obtain the theoretical threshold values—that is, plotting the reciprocal of the concentration against survival time and using the points corresponding to the minimum ct products as the upper limits of the straight lines—does indicate that all the compounds have a time tolerance of 100 ± 5 minutes; that is, an infinitely high concentration requires 100 minutes to kill, if it is assumed that no other factor has entered to change the type of toxic action.

It is also to be noted that the figure obtained by comparison of deguelin with rotenone agrees well with the value obtained in a previous study (4) with another group of goldfish. According to the

formula of Powers (9), $\sqrt{\frac{\tan \theta}{c_0}},^3$ which was used in that study for

comparing toxicity, deguelin had 0.56 the toxicity of rotenone. In the present study, according to the same formula and against a much more resistant group of goldfish, the relative value, or ratio, is nearly the same, 0.52. As determined by a comparison of the slopes of the theoretical velocities of fatality, the ratios are, respectively, 0.79 and 0.75. According to the author's method of comparing minimum ct products, the ratios are 0.55 and 0.61. For a practical comparison, as explained in a previous paper (5), the last figures may be accepted; that is, deguelin has 0.6 the toxicity of rotenone.

Optically active dihydrodeguelin has essentially the same toxicity as rotenone between the concentrations of 0.2 and 0.9 mg per liter. At lower concentrations the latter is increasingly more toxic, this being apparently the result of a lower threshold of toxicity. Its theoretical threshold is about half that of the active dihydrodeguelin.

Optically active dihydrodeguelin is more than twice as toxic as the inactive derivative—2.2 times as toxic according to a comparison of the minimum ct products. The theoretical threshold of toxicity of the inactive derivative is nearly twice as great as that of the active derivative. The minimum survival times, although apparently differing very little, occur in a region of much higher concentration (probably twice or more) in the case of the inactive derivative. However, despite the fact that the inactive form is nearly half as toxic as the *levo* form, it should not be inferred that the *dextro* twin of the latter would be nontoxic. The configuration of the compounds has not been established, and the possession of two asymmetric carbon atoms makes possible several optical isomers. Furthermore, the toxic action of the inactive derivative does not parallel that of a

³ Powers used c instead of c_0 to indicate the threshold of toxicity concentration.

mixture half of which is the active derivative and half a nontoxic derivative. This is shown by replotting the survival-time curve, using half the actual concentrations of the determined points. At very low concentrations the resultant curve approaches very closely that of the active dihydrodeguelin but diverges at high concentrations.

Of interest also is the comparison of deguelin and the active dihydro derivative. According to the ratio of the minimum *ct* products, the latter is 1.33 times as toxic as the former. This may be compared with the value, 1.48, found for a similar structural relationship, in the case of four pairs of optically active rotenone derivatives (6). On the other hand, the inactive dihydro derivative is only 0.60 as toxic as inactive deguelin.

SUMMARY

A study has been made of the toxicity to goldfish of active and inactive dihydrodeguelins, and the results have been compared with each other and with those obtained with rotenone and deguelin. Three criteria were used as the basis of these comparisons—the maximum rate of increase of velocity with increase in concentration, $\tan \theta$; the minimum product of concentration and time (*ct*)_m; and

Powers' formula, $\sqrt{\frac{\tan \theta}{c_0}}$, where *c*₀ is the theoretical threshold of toxicity. According to all these criteria the following descending order of toxicity was found: Rotenone, active dihydrodeguelin, deguelin, and inactive dihydrodeguelin.

Optically active dihydrodeguelin has essentially the same toxicity as rotenone between the concentrations 0.2 and 0.9 mg per liter. At lower concentrations rotenone is increasingly more toxic, approaching a maximum ratio of about 2 to 1.

Active dihydrodeguelin is more than twice as toxic as the inactive derivative. According to a comparison of the minimum *ct* products, the relative value is 2.2.

Active dihydrodeguelin is 1.33 times as toxic as inactive deguelin. This ratio is about the same as that (1.48) of the toxicities for active dihydro derivatives of rotenone to those of their parent compounds.

LITERATURE CITED

- (1) CLARK, E. P.
1931. DEGUELIN. I. THE PREPARATION, PURIFICATION, AND PROPERTIES OF DEGUELIN, A CONSTITUENT OF CERTAIN TROPICAL FISH-POISONING PLANTS. *Jour. Amer. Chem. Soc.* 53: 313-317.
- (2) ———
1932. DEGUELIN. IV. THE STRUCTURE OF DEGUELIN AND TEPHROSIN. *Jour. Amer. Chem. Soc.* 54: 3000-3008.
- (3) GERSDORFF, W. A.
1930. A METHOD FOR THE STUDY OF TOXICITY USING GOLDFISH. *Jour. Amer. Chem. Soc.* 52: 3440-3445, illus.
- (4) ———
1931. A STUDY OF THE TOXICITY OF TOXICAROL, DEGUELIN, AND TEPHROSIN USING THE GOLDFISH AS THE TEST ANIMAL. *Jour. Amer. Chem. Soc.* 53: 1897-1901, illus.
- (5) ———
1935. A NEW CRITERION FOR THE COMPARISON OF TOXICITY WITH RESPECT TO CONCENTRATION AND TIME. *Jour. Agr. Research* 50: 881-891, illus.

- (6) 1935. THE QUANTITATIVE RELATIONSHIP BETWEEN THE CONSTITUTION AND TOXICITY OF SOME ROTENONE DERIVATIVES. *Jour. Agr. Research* 50: 893-898, illus.
- (7) HALLER, H. L., and LaFORGE, F. B.
1934. ROTENONE. XXX. THE NON-CRYSTALLINE CONSTITUENTS OF DERKIS ROOT. *Jour. Amer. Chem. Soc.* 56: 2415-2419.
- (8) LaFORGE, F. B., HALLER, H. L., and SMITH, L. E.
1933. THE DETERMINATION OF THE STRUCTURE OF ROTENONE. *Chem. Rev.* 12: 181-213.
- (9) POWERS, E. B.
1917. THE GOLDFISH (*CARASSIUS CARASSIUS*) AS A TEST ANIMAL IN THE STUDY OF TOXICITY. *Ill. Biol. Monog.* 4, no. 2, 73 pp., illus.

A NEW BACTERIAL SPECIES ISOLATED FROM STRAWBERRIES¹

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INTRODUCTION

In the course of a microbiological investigation of frozen-pack fruits and vegetables made in 1929 and 1930, the same species of bacterium was observed in great numbers in many samples of fresh strawberries going into frozen pack. Samples of berries were studied in 1929 from stations in Norfolk, Va., Fruitland, Md., and Selbyville, Del., and in 1930 at Portsmouth, Va. Plates from all four localities showed this bacterium to be the predominating organism in the fresh berries, whether or not they were washed before samples were taken. This organism occurs principally on the outside of the fruit and, so far as is known, has no bad effect upon the texture, flavor, appearance, or healthfulness of fresh or frozen strawberries.

The methods and media used in this study are those described in the manual of methods of the Society of American Bacteriologists.² One hundred grams of strawberries were placed in a sterile jar, and the berries were mashed thoroughly and mixed with 100 cc of sterile water. Plates were poured from this mixture after the proper dilutions had been made. Several colonies of the organism were selected for cultivation from plates poured in different localities, but as all the isolations reacted to the tests in a uniform way, only one will be described. Plates poured from the water before it was used to wash the berries and from the sugar used in the packs failed to show any colonies of the bacterium. This bacterium is believed to be a new species, and the name *Achromobacter delmarvae* is proposed for it.

DESCRIPTION

MORPHOLOGY

Achromobacter delmarvae, n. sp.

Short, medium-sized rod with rounded ends. Average size 1.5μ by 0.75μ . Occurs as single rod or in pairs, also in short chains. No capsules or spores demonstrated. Nonmotile, Gram-negative, not acid-fast. In old cultures a few rods found slightly larger than the ordinary vegetative size.

CULTURAL CHARACTERS

On beef-infusion agar, pH 7.0, colonies visible in 24 hours at 26° to 31° C. Develop at moderate rate, but always small, rarely over 5 mm in diameter, even when plates are scantily seeded. Colonies circular, raised, with smooth edges, glistening, translucent, bluish-white color in all lights. Internal structure amorphous, surface smooth. Buried colonies lens-shaped. Colonies on gelatin similar to those on agar.

Agar stroke cultures show abundant filiform growth, raised, glistening, smooth, translucent, bluish white, no odor; old cultures slightly viscid; medium unchanged.

Agar stab cultures grow abundantly. Surface growth round, smooth, glistening, bluish white, raised. Filiform growth the whole length of stab, but growth is best at top.

Gelatin stab cultures show scanty growth and no liquefaction after a month.

¹ Received for publication July 24, 1935; issued October 1935. Read at the annual meeting of the American Society of Bacteriologists, Baltimore, Md., December 31, 1931. Abstracted as follows: SMART, H. F. A NEW BACTERIAL SPECIES ISOLATED FROM STRAWBERRIES. Jour. Bact. 23: 41-42. 1932.

² SOCIETY OF AMERICAN BACTERIOLOGISTS. COMMITTEE ON BACTERIOLOGIC TECHNIC. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. . . 46 pp. and leaflets, illus. Geneva, N. Y. 1930. 4p.]

Nutrient broth cultures show good clouding in 24 hours. Delicate white pellicle formed in 5 days. Precipitate abundant, white, and slightly stringy. No odor; color of medium unchanged:

Cultures on potato cylinders produce abundant growth, grayish white, glistening, smooth, raised. Medium changed from white to smoke-gray (Ridgway, pl. 46).³

PHYSIOLOGY

Optimum temperature for growth 26° C., good growth up to 31°. Growth at 37° very slight. Organisms remain alive at -8°; do not grow abundantly after being held at that temperature for any considerable length of time.

Optimum hydrogen-ion concentration about pH 7.0.

Bacterium white, but has slightly bluish color on agar and gelatin, probably caused by its translucent qualities. No color in broth, but on potato cylinders growth definite gray-white.

Indole not produced in tryptophane broth in 10 days.

Hydrogen sulphide and ammonia not produced in beef broth in 10 days.

Diastatic action of bacterium weak. Starch agar plates inoculated and tested with iodine solution; in 5 days very slight, clear zone of about 2 mm around the streak. Plates incubated for longer periods failed to show any wider clear zone.

Nitrates reduced in 7 days at 26° C. in medium containing 0.1 percent Difco peptone and 1 percent potassium nitrate.

Fermentation tubes used to study relation of organism to oxygen. Medium used was beef broth with 1 percent of the following sugars: Glucose, lactose, sucrose, mannitol, and glycerol. Growth in closed arm of all tubes but heavier in open end, showing bacterium to be facultative anaerobe.

Growth slow in sterile milk; acid curd formed in 12 to 14 days. Milk turned decided chocolate brown, beginning at top. Peptonization does not take place in 2 months.

Litmus in milk turns pink first and is entirely reduced in 5 days; pink color returns in 10 days, after which curd forms and browning of medium starts, as in the plain milk, in 12 to 14 days.

With bromocresol purple as indicator in fermentation tubes with beef-extract sugar broths, bacterium gives acid reaction after 5 days in glucose, lactose, glycerin, and mannite, and alkaline reaction in the same time in sucrose. No gas formed in any of the tubes.

SUMMARY

A bacterial species isolated from fresh strawberries is described.⁴ This organism was found to be the predominating species in samples of berries collected from four localities in Delaware, Maryland, and Virginia over a period of 2 years. The species is thought to be one hitherto undescribed, and the name *Achromobacter delmarvae* is proposed.

The organism is a nonmotile, Gram-negative, nonspore-forming, short rod; average size, 1.5 μ by 0.75 μ ; agar colonies are round, raised, glistening, translucent, bluish white, smooth, entire margin, amorphous; agar stroke cultures are filiform, raised, glistening, smooth, translucent, bluish white, no odor, medium unchanged, gelatin not liquefied; pellicle and stringy white precipitate in beef broth; grayish white on potato.

The organism grows best at 26° to 31° C.; is weakened at 37° and -8° C.; does not form indole, hydrogen sulphide, or ammonia; diastatic action is weak; nitrates are reduced; aerobic growth better than anaerobic; acid curd formed in 12 to 14 days; color of milk becomes chocolate-brown; reduction of litmus in 5 days in litmus milk with curd formation and browning as in plain milk; forms acid and no gas in glucose, lactose, glycerin, and mannite; alkaline reaction and no gas in sucrose.

³ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912

⁴ Other species of bacteria, yeasts, and molds isolated from fresh and frozen fruits and vegetables are listed in the following article: SMART, H. F. MICRO-ORGANISMS SURVIVING THE STORAGE PERIOD OF FROZEN PACK FRUITS AND VEGETABLES. Phytopathology 24: 1319-1331. 1934.

CHEMICAL COMPOSITION OF CANNED PEAS OF TWO VARIETIES OF DIFFERENT SIZES AND GRADES¹

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INTRODUCTION

Canned peas, like other foods, are graded according to "quality", that is, flavor, tenderness, etc., rather than according to the nutritional value of their chemical constituents. Chemical analysis is of prime importance, however, in supplementing the organoleptic tests by providing a sound basis for the selection of food from a nutritional point of view.

Data on the mineral composition of different commercial grades of peas are very inadequate. Previous investigations include papers by Diggs (3),² Boswell (2), and Sayre, Willaman, and Kertesz (8). These investigators studied the chemical changes in peas which are associated with the tenderness of the canned product, and reported that a high calcium content was associated with a tough pea. They found that potash fertilizers tended to reduce toughness and correspondingly to lower the calcium content. Conversely, calcium chloride applied to the soil made peas tougher. Percentages of dry matter, calcium, and total nitrogen were found to increase with size when calculated on the wet basis, but remained practically constant on the dry basis. In a later paper Kertesz (6) reported that the alcohol-insoluble solids (starch, dextrin, hemicelluloses, protein, and fiber) increase with maturity and hence serve as a good index of quality.

Because of the increased interest in the mineral content of foods, especially of the elements calcium, phosphorus, iron, copper, and manganese, it was considered desirable to obtain data on the variation in these elements in the commercial grades of canned peas. Data are also presented regarding the fiber, protein, and dry-matter content of the various sizes that are on the market.

MATERIAL AND METHODS

The canned peas used in this work were obtained from four different canneries, located in various parts of Wisconsin. By such a selection it was thought that the samples would be somewhat representative of the canned products of the State.

The can and contents were weighed, the liquor was drained off, and the can reweighed. The can was then emptied of the drained peas and weighed again. The liquor and drained peas were combined and dried in a steam oven in large evaporating dishes which were covered with heavy wrapping paper to prevent any material from dropping into the samples. They were then ground in an iron spice

¹ Received for publication Jan. 15, 1935; issued October 1935.

² Reference is made by number (italics) to Literature Cited, p. 370.

mill to a fine powder.³ From 5 to 13 cans of each size were treated in this way and a composite sample was formed by mixing equal portions of the individual samples. From this mixture aliquots were taken for the different determinations.

Nitrogen was determined by the Kjeldahl method (1) and calculated as protein $N \times 6.25$, phosphorus by the volumetric method of the Association of Official Agricultural Chemists (1), calcium by a modified McCrudden (7) method, copper by the method of Elvehjem and Lindow (4), manganese by the method of Skinner and Peterson (9), and iron by the method of Kennedy (5).

RESULTS AND DISCUSSION

Table 1 gives the variations in pea and liquor content per can and also the variations in the dry matter of the samples.

One hundred and twenty-seven samples, 52 of the Alaska or smooth variety, and 75 of the sweet or wrinkled variety, were used. The Alaska peas were of four different sizes, ranging from the smallest, no. 1, to the largest, no. 4. The sweet or wrinkled consisted of six different sizes ranging from no. 1 to no. 6 and an extra lot of ungraded peas of sizes 1 to 6. In any one size of canned peas there was found to be only a small variation in the weight of the total contents of similar cans, the maximum difference being 3.2 percent. However, there was considerable variation in the liquor and pea content of the various cans of each size, the maximum difference in weight of liquor being 19.5 percent and of the peas, 13.6 percent.

TABLE 1.—*Variation in quantity contained in can, and in dry matter of canned product, for Alaska and for an unknown sweet or wrinkled variety of peas, packed in no. 1 and no. 2 cans*

ALASKA

Pea size no.	Content	Weight of contents								Percentage of dry matter	
		Total		Drained peas		Liquor		Dry matter			
		No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can
1	High	Grams 316	Grams 601	Grams 205	Grams 374	Grams 132	Grams 252	Grams 43	Grams 72	13.6	12.0
	Low	314	594	183	342	110	223	34	63	10.7	10.6
	Average 1	315	597	197	361	118	236	38	68	12.1	11.4
2	High	323	604	215	400	120	223	48	103	14.9	17.0
	Low	319	597	200	381	104	204	41	84	12.8	13.9
	Average 1	321	602	207	390	113	212	46	99	14.0	15.9
3	High	324	608	220	396	128	234	57	112	17.8	18.4
	Low	317	603	190	369	103	211	52	107	16.0	17.6
	Average 1	320	606	207	380	113	226	54	110	16.8	18.0
4	High	325	607	211	410	118	226	62	123	19.0	20.4
	Low	321	596	204	370	114	193	59	105	18.1	17.5
	Average 1	323	603	207	390	116	211	60	116	18.6	19.2

¹ Each figure is the average of 6 or 7 cans.

³ The iron content of samples ground in a spice mill was from 22 to 65 percent higher than that of samples ground in a mortar. Only the latter method of grinding was used in the case of the iron analyses.

TABLE 1.—Variation in quantity contained in can, and in dry matter of canned product, for Alaska and for an unknown sweet or wrinkled variety of peas, packed in no. 1 and no. 2 cans—Continued

SWEET OR WRINKLED, VARIETY UNKNOWN

Pea size no.	Content	Weight of contents								Percentage of dry matter	
		Total		Drained peas		Liquor		Dry matter			
		No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can
		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams		
	High	311	594	189	358	129	240	30	73	9.6	12.4
	Low	310	575	181	347	122	226	29	56	9.3	9.7
	Average ¹	311	587	185	353	126	235	30	63	9.4	10.7
2	High	319	600	205	403	119	229	39	71	12.2	11.8
	Low	313	582	199	353	111	194	36	56	11.4	9.6
	Average ¹	316	596	202	384	115	212	38	65	11.8	11.0
3	High	324	602	212	400	124	231	46	81	14.2	13.5
	Low	315	595	195	364	112	198	40	66	12.7	11.0
	Average ¹	320	599	202	384	118	214	43	76	13.3	12.7
4	High	320	598	210	384	115	226	52	89	16.4	14.8
	Low	316	586	205	360	108	214	49	76	15.3	12.9
	Average ¹	318	593	206	373	111	219	50	83	15.7	13.9
5	High	325	599	205	386	127	230	55	103	16.9	17.2
	Low	317	592	198	368	113	212	51	79	16.2	13.3
	Average ²	320	596	202	377	118	219	53	96	16.5	16.0
6	High	608	-----	408	-----	239	-----	107	-----	17.8	-----
	Low	595	-----	358	-----	195	-----	92	-----	15.3	-----
	Average ¹	601	-----	391	-----	210	-----	102	-----	17.0	-----
Ungraded	High	603	-----	374	-----	230	-----	90	-----	14.9	-----
	Low	576	-----	369	-----	203	-----	79	-----	13.7	-----
	Average ¹	592	-----	372	-----	218	-----	86	-----	14.5	-----

Each figure is the average of 4, 7, or 9 cans, with the exception of size 1, no. 1 can (311 g), which includes only 2 samples.

Dry matter increased with size of pea. The percentage for the Alaska variety ranged from 11.4 for size 1 to 19.2 for size 4, and for the sweet or wrinkled from 10.7 for size 1 to 17.0 for size 4. This is in agreement with the work of Sayre, Willaman, and Kertesz (8).

It will be seen from figure 1 that on the wet basis the percentages of protein, calcium, phosphorus, and crude fiber increased with increase in size of pea. Also, that in each case the Alaska variety contained the higher percentage of each constituent. However, the increase of these constituents with size of pea is not preferential increase with respect to total solids; on the dry basis the percentage of these constituents remains practically constant, i. e., mineral elements and total solids are laid down at approximately the same rate. The data for protein and calcium in relation to size are in agreement with those reported by Sayre et al. (8).

Figure 2 shows the iron, copper, and manganese content of the peas calculated on the wet basis. The general trend was an increase with size, except in the case of copper in the Alaska variety; here there was a decrease. Again the composition of the Alaska variety was found to be different from that of the sweet or wrinkled variety.

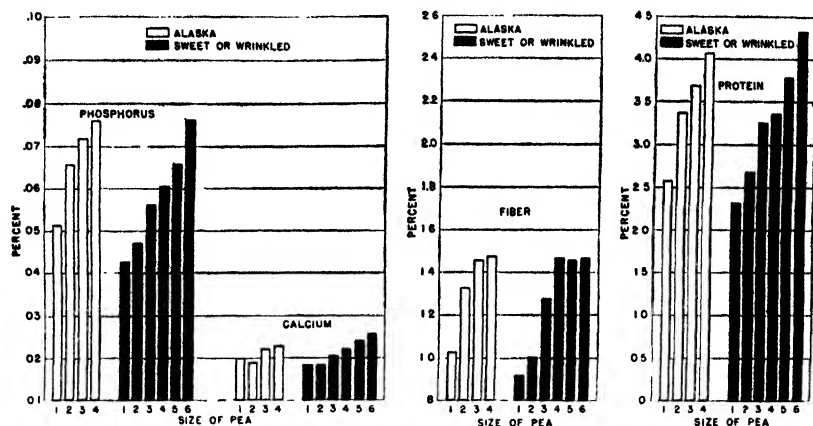


FIGURE 1.—Protein, calcium, phosphorus, and crude fiber content of canned peas (wet basis).

Because of a larger amount of dry matter and hence a larger sum of all the constituents, the larger pea has the greater food value.

The question arose as to whether such factors as soil, fertilizer treatment, inoculation, climatic conditions, etc., might have such an effect on the composition of the peas as to mask the variation due to size. In order to minimize these factors, samples were obtained from

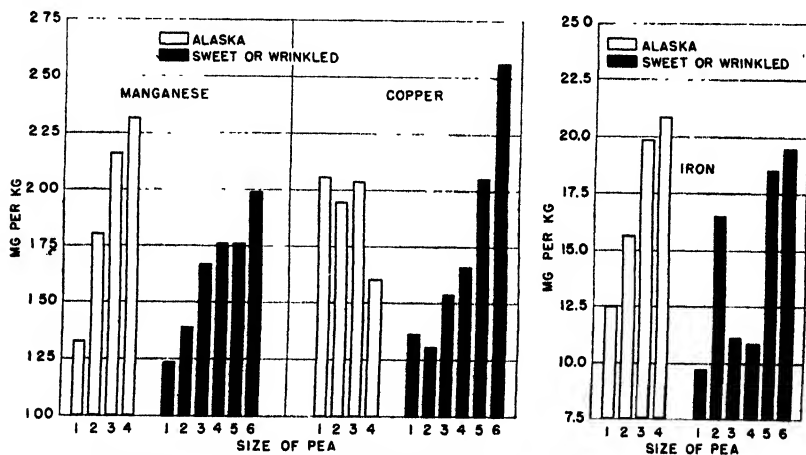


FIGURE 2.—Iron, copper, and manganese content of canned peas (wet basis).

a single cannery and their composition determined. In table 2 it is shown that the chemical composition of the peas from 1 cannery is very similar to that of the composite samples from 4 canneries. The variables, therefore, which affect the constituents of the canned product appear to have been practically the same for all the canneries.

TABLE 2.—Protein, phosphorus, and calcium content of sweet or wrinkled and Alaska peas from 1 cannery and of composite samples from 4 canneries ¹ (wet basis)

Size no.	Variety and grade	Protein		Phosphorus		Calcium	
		A	B	A	B	A	B
	Sweet or wrinkled, unknown:	Percent	Percent	Percent	Percent	Percent	Percent
1	Standard.....	2.22	2.43	0.0431	0.0418	0.0173	0.0202
2	Fancy.....	2.63	2.71	.0447	.0494	.0166	.0205
3	Extra standard.....	3.34	3.08	.0598	.0525	.0186	.0228
4	Standard.....	3.06	3.66	.0578	.0621	.0193	.0252
5	Do.....	3.96	3.63	.0721	.0595	.0228	.0249
6	Do.....	4.56	4.10	.0648	.0669	.0250	.0273
	Alaska:						
1	Extra standard.....	2.47	2.68	.0500	.0528	.0193	.0209
2	Standard.....	3.68	3.03	.0752	.0555	.0187	.0190
3	Do.....	3.83	3.49	.0799	.0639	.0234	.0212
4	Do.....	4.28	3.82	.0910	.0710	.0216	.0243

¹ A, figures for composite samples (5 cans each) from 1 cannery; B, figures for composite samples, (4 or 8 cans each) from 4 canneries

As there are variations in quality within a given size of pea the question arose as to the relation between grade and composition when all samples were taken from one size. While there are differences in composition the figures in table 3 do not vary in any regular manner. In the Alaska variety the Standard grade contains the largest amount of dry matter, in the wrinkled variety the smallest amount. The protein content follows that of dry matter but the other constituents are quite constant.

TABLE 3.—Variation in composition of Alaska and sweet or wrinkled peas of different grades (dry basis)

Variety and grade ¹	Dry matter	Protein	Phosphorus	Calcium	Iron	Manganese	Copper
	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Alaska, size 2 ²							
Standard.....	16.88	3.68	0.0752	0.0187	0.01010	0.00115	0.00125
Extra standard.....	16.06	3.08	.0561	.0210	.00943	.00126	.0010
Fancy.....	14.29	3.06	.0537	.0167	.01250	.00151	-----
Sweet or wrinkled, unknown, size 4: ²							
Standard.....	13.37	2.98	.0542	.0216	-----	-----	-----
Extra standard.....	15.92	4.04	.0691	.0218	-----	-----	-----
Fancy.....	14.04	3.28	.0495	.0255	-----	-----	-----

¹ As given by the packer.

² Average of 5 samples of each grade.

SUMMARY

The protein, calcium, phosphorus, copper, iron, manganese, and crude fiber content of canned peas of different sizes are given. The data are based on 127 samples from four canneries and represent the two important varieties of peas, smooth and wrinkled.

The percentages of dry matter, protein, calcium, phosphorus, copper, iron, manganese, and crude fiber of the canned product in general increased with size of pea. An exception is the copper content of the Alaska variety. If calculated on the dry basis the protein, calcium, and other constituents are found to be rather constant.

From certain points of view the nutritive value of the Alaska variety was superior to that of the sweet or wrinkled as the former contained more protein, calcium, and phosphorus.

LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Ed. 3, 593 pp., illus. Washington, D. C.
- (2) BOSWELL, V. R.
1929. FACTORS INFLUENCING YIELD AND QUALITY OF PEAS—BIOPHYSICAL AND BIOCHEMICAL STUDIES. Md. Agr. Expt. Sta. Bull. 306, pp. 341-382.
- (3) DIGGS, J. C.
1914. ANALYSES SHOWING THE COMPOSITION OF THE DIFFERENT GRADES OF COMMERCIAL PACK PEAS. Jour. Indus. and Engin. Chem. 6: 310-313.
- (4) ELVEHJEM, C. A., and LINDOW, C. W.
1929. THE DETERMINATION OF COPPER IN BIOLOGICAL MATERIALS. Jour. Biol. Chem. 81: 435-443.
- (5) KENNEDY, R. P.
1927. THE QUANTITATIVE DETERMINATION OF IRON IN TISSUES. Jour. Biol. Chem. 74: 385-391, illus.
- (6) KERTESZ, Z. I.
1934. NEW OBJECTIVE METHODS TO DETERMINE MATURITY OF CANNED PEAS. CHEMICAL METHOD REVEALS SUBSTANDARD AND SOAKED PEAS. Food Indus. 6: 168-170.
- (7) MELOCHE, V. W., CLIFCORN, L. E., and GRIEM, W. B.
1933. DETERMINATION OF CALCIUM IN MINERAL MIXTURES. Jour. Assoc. Off. Agr. Chem. 16: 240-245.
- (8) SAYRE, C. B., WILLAMAN, J. J., and KERTESZ, Z. I.
1931. FACTORS AFFECTING THE QUALITY OF COMMERCIAL CANNING PEAS. N. Y. Agr. Expt. Sta. Tech. Bull. 176, 76 pp., illus.
- (9) SKINNER, J. T., and PETERSON, W. H.
1930. THE DETERMINATION OF MANGANESE IN ANIMAL MATERIALS. Jour. Biol. Chem. 88: 347-351.

PUBESCENT AND GLABROUS CHARACTERS OF SOYBEANS AS RELATED TO RESISTANCE TO INJURY BY THE POTATO LEAF HOPPER¹

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INTRODUCTION

Soybeans (*Soja max* (L.) Piper) may be divided roughly into the following three groups, on the basis of amount and type of pubescence on the leaves, stems, and pods: (1) Practically glabrous, (2) sparingly appressed-hairy, and (3) densely rough-hairy (fig. 1). The varieties grown commercially in the United States are practically all of the rough-hairy type, while many of the recent introductions from the Orient are glabrous or appressed-hairy. According to W. J. Morse,³ these types, because of their resistance to the attack of a pod-boring insect, are widely grown in the Orient. When planted at the Arlington Experiment Farm, Rosslyn, Va., the glabrous type was heavily infested and seriously injured by the potato leaf hopper, *Empoasca fabae* (Harris); while rough-hairy varieties, grown nearby and presumably exposed to the same opportunities for infestation, were relatively free from leaf hoppers and from symptoms of leaf hopper injury. This difference is illustrated in figure 2.

Hollowell, Monteith, and Flint (1),⁴ Pieters (9), and Monteith and Hollowell (6) have called attention to a similar situation in the case of red clover. The last-named writers report that—

In red clover there is a direct correlation between hairiness of the leaves, petioles and stems and resistance to hopper injury. All the European and South American red clovers imported into this country (susceptible) are smooth or appressed pubescent, whereas red clover produced in this country and Canada (resistant) is rough pubescent.

Poos and Smith (10) compared the oviposition of *Empoasca fabae* females on red clover and soybean plants with the various types of pubescence and found that more nymphs hatched from the glabrous and appressed-hairy types than from the rough-hairy. The preference of the females for these types for oviposition is doubtless one reason for their greater injury when all three types are grown together in a comparative field test.

¹ Received for publication June 1, 1935; issued October 1935. This paper reports the results of certain phases of a cooperative study of the injury caused by *Empoasca fabae* (Harris) to forage legumes, which is being made at Arlington Experiment Farm, Rosslyn, Va., by the Division of Cereal and Forage Insects, Bureau of Entomology and Plant Quarantine, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

² Indebtedness is acknowledged to C. M. Woodworth, Illinois Agricultural Experiment Station, and W. J. Morse, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for supplying the seed used in these experiments; and also to F. W. Poos, Division of Cereal and Forage Insects, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for infesting field cages with *Empoasca*.

³ Oral communication.

⁴ Reference is made by number (italic) to Literature Cited, p. 380.

Two genetically distinct types of glabrousness have been demonstrated in soybeans: Nagai and Saito (7) in 1923 discovered a type in which glabrousness was dominant, while Stewart and Wentz (12) in 1926 reported a type in which glabrousness was recessive. In crosses with pubescent varieties, the ratio is a 3:1 in each case, show that a single-factor pair is involved. It appeared that a study of a large number of progenies from a cross between a pubescent and a

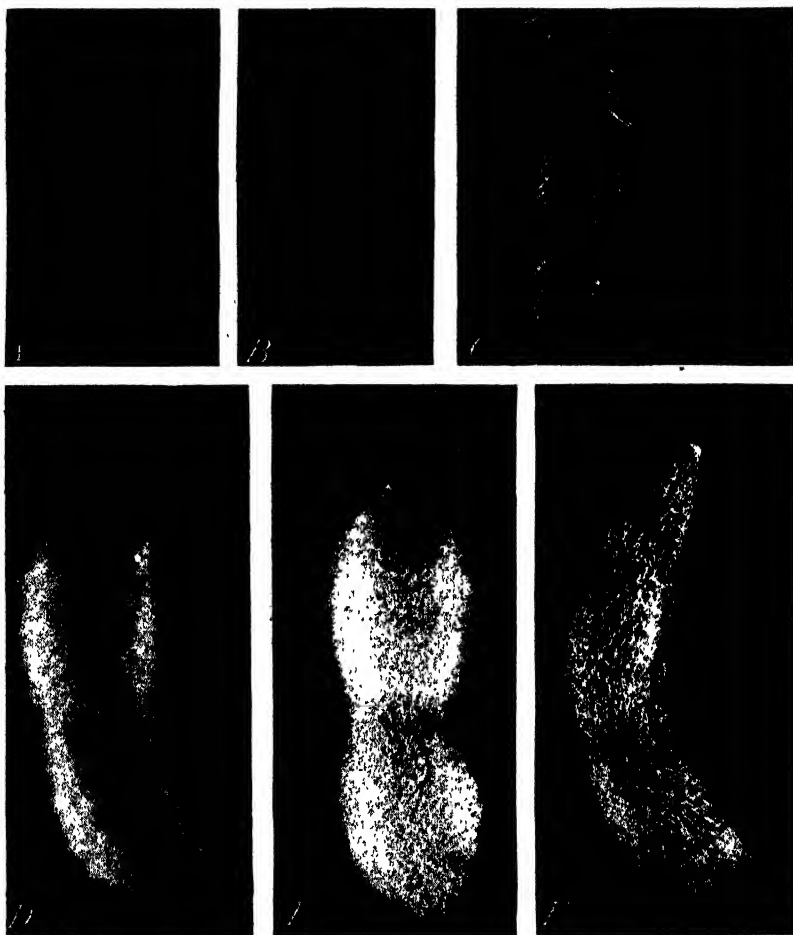


FIGURE 1.—Stems and petioles (A-C) and pods (D-F) of practically glabrous (A, D), sparingly appressed-hairy (B, E), and densely rough-hairy (C, F) soybeans. A-C, $\times 3$; D-F, $\times 4$.

glabrous soybean would yield evidence as to whether the rough-hairy pubescence or some other character of the pubescent plants was responsible for their resistance to injury by *Empoasca fabae*. Seed of 29 glabrous F_2 plants from a cross made between Illini (rough-hairy) and a dominant glabrous soybean were obtained from C. M. Woodworth of the Illinois Agricultural Experiment Station. This paper reports the results from a study of the F_3 progenies of these 29 plants grown at Arlington Experiment Farm in 1931, of 108 F_4

progenies grown in 1932, and of 73 F_2 progenies grown in 1933. Included also are observations on 34 glabrous and appressed-pubescent soybean introductions grown in 1931 and 32 similar introductions grown in 1932.

EXPERIMENTAL METHODS AND RESULTS

SOYBEAN PROGENIES

Seed of 29 glabrous F_2 plants from a cross between Illini (rough-hairy) and a dominant glabrous soybean was sown in progeny rows May 20, 1931. An excellent stand was secured and by July 13 marked differences in *Empoasca* infestation, stunting of growth, and injury to the leaves were evident between the glabrous and the rough-hairy individuals. The notes taken on this date may be summarized as follows: (1) Of the 29 F_2 progenies, 14 were homozygous glabrous



FIGURE 2.—Alternate rows of rough-hairy (Dixie) and glabrous (S. P. I.¹ no. 55069) soybeans growing at Arlington Experiment Farm on Aug. 6, 1931. The rough-hairy plants in the outside rows are practically free from *Empoasca* and show no symptoms of injury, while the glabrous plants in the center row are heavily infested and severely stunted and have curled leaves with yellowed necrotic margins.

and 15 segregated in a ratio of approximately 3 glabrous to 1 rough-hairy; (2) the glabrous individuals (total number 813), in both the homozygous and the heterozygous progenies, were all heavily infested with *Empoasca* and were severely stunted in growth, averaging only 8 inches in height, and their leaves were curled and had yellowed necrotic margins; (3) the rough-hairy individuals (total number 129) were all relatively free from *Empoasca* and averaged 15 to 16 inches in height, and their leaves showed no symptoms of leaf hopper injury.

These differences in degree of *Empoasca* infestation, stunting of growth, and leaf injury persisted throughout the season, and when final notes were taken on September 2 the glabrous plants averaged about 13 inches in height and bore relatively few seed pods, while the rough-hairy plants averaged about 25 inches in height and bore abundant seed. Late in September 1931, seed was harvested from individual glabrous and rough-hairy plants.

¹ S. P. I. denotes Seed and Plant Introduction, Bureau of Plant Industry.

Seed of 108 of the F_3 plants was sown in progeny rows on May 23, 1932, and, as in the previous season, by the middle of July marked differences in *Empoasca* infestation, stunting of growth, and injury to the leaves were evident between the glabrous and the rough-hairy individuals. The notes taken between July 19 and 27, 1932, may be summarized as follows: (1) Of the 108 F_4 progenies, 47 were homozygous glabrous, 32 segregated in a ratio of approximately 3 glabrous to 1 rough-hairy, and 29 were homozygous rough-hairy; (2) the glabrous individuals (total number 4,509), in both the homozygous glabrous and the heterozygous progenies, were all heavily infested with *Empoasca* and were stunted in growth, averaging about 15 inches in height, and their leaves were curled and were beginning to yellow at the margins, although little marginal necrosis had developed at this time; (3) the rough-hairy individuals (total number 5,449) were all relatively free from *Empoasca* and averaged 25 inches in height, and their leaves showed no symptoms of leaf hopper injury.

As the season advanced, marginal leaf necrosis became much more pronounced on the glabrous plants, and the differences in degree of *Empoasca* infestation and stunting of growth persisted. Late in September 1932, seed was harvested from individual plants in each progeny.

Seed of 73 of the F_4 plants was sown in progeny rows on May 24, 1933. By the middle of July, as in the two previous seasons, marked differences in *Empoasca* infestation, stunting of growth, and injury to the leaves were evident between the glabrous and the rough-hairy individuals. These differences, which are typical of those present in each of the three seasons, are shown in figure 3, illustrating the three types of F_5 progenies. As the season advanced the differences became even more pronounced, and the notes taken September 25, 1933, may be summarized as follows: (1) Of the 73 F_5 progenies, 32 were homozygous glabrous, 32 segregated in a ratio of approximately 3 glabrous to 1 rough-hairy, and 9 were homozygous rough-hairy; (2) the glabrous individuals (total number 3,248), in both the homozygous glabrous and the heterozygous progenies, were all infested with *Empoasca* and were stunted in growth, averaging about 14 inches in height, and their leaves were curled and had yellowed necrotic margins; (3) the rough-hairy individuals (total number 1,177) were all relatively free from *Empoasca* and averaged about 29 inches in height, and their leaves showed no symptoms of leaf hopper injury.

The data and notes on the three generations of progenies from a cross between Illini (rough-hairy) and a dominant glabrous soybean, which were grown in 1931, 1932, and 1933, are summarized in table 1. The consistency with which over a period of three generations in material of known genetic constitution the glabrous plants all were heavily infested and severely stunted and had curled hopper-burned leaves, while all the rough-hairy plants were practically free from *Empoasca*, grew vigorously, and had normal leaves with uninjured margins, appears to justify the conclusion that, in the soybeans tested, resistance to the injury caused by the potato leaf hopper was correlated with the occurrence of rough-hairy pubescence.



FIGURE 3.—Homozygous glabrous (A), heterozygous (B), and homozygous rough-hairy (C) F_2 progenies from a cross between Illini (rough-hairy) and a dominant glabrous soybean, growing at Arlington Experiment Farm on July 14, 1933. The glabrous plants are heavily infested with *Empoasca*, are severely stunted, and have curled leaves with yellowed necrotic margins; while the rough-hairy plants are practically free from leaf hoppers and are growing vigorously, and their leaves show no symptoms of hopper injury. The background is ruled in 1-foot squares.

TABLE 1.—Summarized data on 3 generations of soybeans from a cross between Illini (rough-hairy) and a dominant glabrous type, grown at Arlington Experiment Farm, in 1931, 1932, and 1933 to determine whether resistance to injury by the potato leaf hopper is correlated with occurrence of rough-hairy pubescence

Year	Generation	Phenotypes	Progenies	Individuals		
				Glabrous ¹	Rough-hairy ²	Ratio of glabrous to rough-hairy
			Number	Number	Number	
1931.....	F ₂	Glabrous.....	14	412	0	
		Glabrous and rough-hairy.....	15	401	129	3.1 : 1
1932.....	F ₁	Glabrous.....	47	3,093	0	
		Glabrous and rough-hairy.....	32	1,416	442	3.2 : 1
1933.....	F ₂	Rough-hairy.....	29	0	5,007	
		Glabrous.....	32	1,995	0	
		Glabrous and rough-hairy.....	32	1,253	422	2.97 : 1
		Rough-hairy.....	9	0	755	
		Total.....	210	8,570	6,755	

¹ Heavily infested with *Empoasca* and severely stunted, having curled leaves with yellowed necrotic margins.

² Practically free from *Empoasca*, vigorous, having normal leaves with uninjured margins.

SOYBEAN INTRODUCTIONS

Seven glabrous and 27 appressed-hairy soybean introductions were grown in rod rows adjacent to the F₂ progenies in 1931, while 16 glabrous and 16 appressed-hairy introductions were grown adjacent to the F₄ progenies in 1932. The rough-hairy varieties Herman and Dixie were included in the planting of both years for comparison. In both 1931 and 1932, the glabrous introductions (fig. 4, A) were heavily infested with *Empoasca*, were stunted in growth to about 1 foot in height, and had curled leaves with yellowed necrotic margins. The appressed-hairy introductions (fig. 4, B), on the other hand, were less heavily infested, and, while their leaves were crinkled and slightly yellowed at the margins, the plants attained a final height of 2 feet or more without evidence of marked stunting. The rough-hairy varieties attained a height of approximately 2½ feet each season and were practically free from leaf hoppers, and their leaves showed no symptoms of hopper injury.

Three of the glabrous introductions grown in 1931 and eight of those grown in 1932 contained a few rough-hairy plants, apparently as a result of segregation. In all such instances the glabrous plants were heavily infested and stunted while the rough-hairy plants were practically free from leaf hoppers and grew normally (fig. 4, C) just as in the case of the segregating progenies discussed above. The possibility of establishing leaf hopper resistant strains by selection of the hairy plants from these segregating introductions is evident. By this method, Kottur (4) and Kottur and Maralihalli (5) established a leaf hopper resistant strain of American cotton (Gadag No. 1) which almost entirely replaced the ordinary Dharwar-American variety (a mixture of hairy, sparingly hairy, and glabrous phenotypes) in the Dharwar-American tract in India.

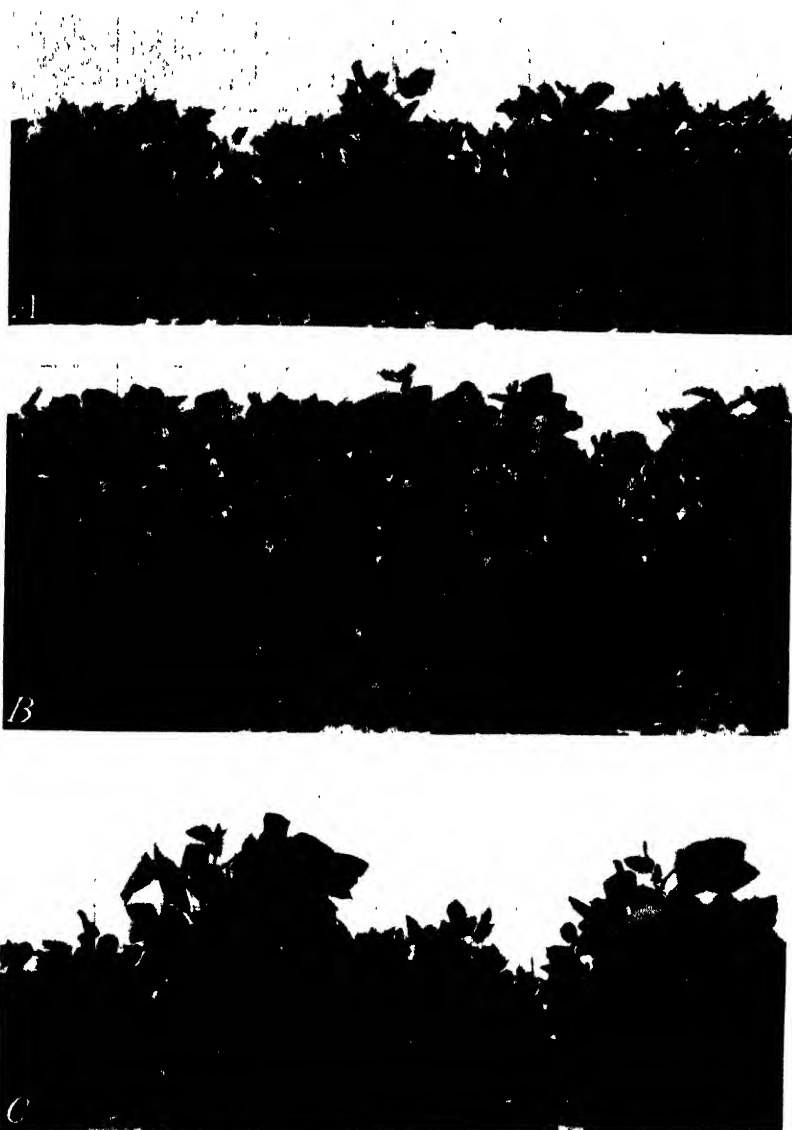


FIGURE 4.—Glabrous (A), appressed-hairy (B), and segregating (C) soybean introductions, growing on July 29, 1932. The glabrous plants have been stunted by a heavy infestation of leaf hoppers, the appressed-hairy plants are less heavily infested and show no marked stunting, although their leaves are crinkled and slightly yellowed at the margins. The rough-hairy plants in C are practically free from leaf hoppers, and their leaves show no symptoms of injury. The background is ruled in 1-foot squares.

DISCUSSION

Nagai and Saito (7), Stewart and Wentz (12), Owen (8), Woodworth and Veatch (15), and Veatch (13) have reported that glabrous soybeans were stunted in growth and yielded less than the pubescent varieties. Veatch (13) concluded from his genetic studies that in the dominant glabrous type of soybean, the factor P_1 inhibits the normal



FIGURE 5.—Representative plants of three F_1 progenies from a cross between Illini (rough-hairy) and a dominant glabrous soybean, grown in a tobacco-cloth cage infested with *Empoasca* (A-D) and in a similar uninfested cage (E-H). The infested glabrous plants (homozygous, A; heterozygous, C) are stunted and have curled leaves with yellowed margins, while those kept free from leaf hoppers (homozygous, E; heterozygous, G) have uninjured leaves and have made practically as tall growth as the rough-hairy plants (homozygous, B, F; heterozygous, D, H). The background is ruled in 1-foot squares.

vigor and plant development as well as pubescence or is closely linked with factors that reduce vigor and development. Since P_1 is not present in the recessive glabrous type, Woodworth (14) concludes that here lack of vigor most likely is due to the recessive factor p_2 , which is responsible for glabrousness in this strain. The writers have observed that when progenies are grown to the young plant stage in a greenhouse free from leaf hoppers, the rough-hairy seedlings grow

more rapidly and somewhat more vigorously than the glabrous seedlings, apparently because of some genetic difference. However, when progenies are grown to maturity out of doors, but under cages so that the plants are kept practically free from leaf hoppers, the glabrous plants, while much lower in seed yield, are not severely stunted in growth. This fact is illustrated by figure 5, showing plants from the F_1 generation of the cross between Illini (rough-hairy) and a dominant glabrous soybean, which were grown in tobacco-cloth cages in 1932. The plants above (A-D) are from a cage containing half of each of three phenotypically different progenies, which were artificially infested with potato leaf hoppers. The plants below (E-H) are from a cage containing the other halves of the same progenies, which were kept free from leaf hoppers throughout the season. The infested glabrous plants (A, C) are stunted and have curled leaves with yellowed margins, while those kept free from leaf hoppers (E, G) have uninjured leaves and are practically as tall as the rough-hairy plants. It would appear, therefore, that the extreme differences in growth and vigor between the rough-hairy and glabrous types when grown in the field are due in part to infestation of the glabrous plants with potato leaf hoppers.

The significance of the dense rough-hairy pubescence in the resistance of this type to leaf hopper injury is an interesting question which so far has been answered only in part. Monteith and Hollowell (6) observed that plants with succulent and fleshy veins or petioles, such as cowpeas, seem to be preferred by the hoppers, and conclude that, although dense pubescence evidently serves as a mechanical obstruction which in some way interferes with the activities of the insects, there appears to be some factor involved other than or in addition to that of hairiness. Poos and Smith (10) compared oviposition and nymphal development of *Empoasca fabae* on plants with the various types of pubescence and concluded that factors other than amount and type of pubescence probably are responsible, at least in part, for the resistance to injury by this leaf hopper observed in some of the strongly pubescent varieties of legumes. Jewett (2) observed no very direct relation between amount of injury and amount of pubescence in red clover and considered some unknown factor to be responsible for the resistance of the native rough-hairy type. He later (3) reported that leaves of Kentucky red clover (rough-hairy) were more resistant to puncturing than leaves of Italian red clover (practically glabrous) and concluded that the greater resistance of Kentucky clover to injury by *E. fabae* is due, in part at least, to greater resistance to puncturing. Since the histological work of Smith and Poos (11) showed that *E. fabae* feeds of necessity upon the vascular tissues of its hosts, measurements of the resistance of the leaf blades of red clover to mechanical puncturing probably are of only slight value in explaining differences in resistance of strains with the various types of pubescence. The results reported in this paper suggest that in the soybeans tested the mechanical protection of dense rough-hairy pubescence was primarily responsible for the resistance of the hairy plants to injury. Otherwise, one must assume the existence of some other character the inheritance of which is controlled by the same hereditary complex as pubescence. If another character for resistance existed, its presence probably would have become evident in at least a few of the segregating progenies.

SUMMARY

Soybean progenies of the F_3 , F_4 , and F_5 generations from a cross between Illini (rough-hairy) and a dominant glabrous soybean were grown in 1931, 1932, and 1933 to determine whether the resistance of rough-hairy soybeans to the injury caused by the potato leaf hopper, *Empoasca fabae* (Harris), is correlated with the occurrence of rough-hairy pubescence in material of known genetic constitution.

In the three generations tested the glabrous individuals of both the homozygous glabrous and the heterozygous progenies were all heavily infested with *Empoasca*, severely stunted in growth, and had curled leaves with yellowed necrotic margins. The rough-hairy individuals, on the other hand, were almost entirely free from *Empoasca*, and grew vigorously, and their leaves showed no symptoms of leaf hopper injury.

Glabrous and appressed-hairy soybean introductions from the Orient were grown adjacent to the progenies in 1931 and 1932. The glabrous plants were heavily infested with leaf hoppers and were severely stunted, while the appressed-hairy plants were less heavily infested and were not markedly stunted in growth, although many of their leaves were crinkled and had yellowed margins which later became necrotic. Some of the glabrous introductions contained rough-hairy plants, probably segregates, which were practically free from leaf hoppers and showed no symptoms of injury.

It would appear that in the soybeans tested resistance to leaf hopper injury was due to the rough-hairy pubescence or to some character the inheritance of which is controlled by the same hereditary complex as pubescence. No evidence of the existence of such a character was obtained in these studies, which involve three generations of progenies consisting of 8,570-glabrous and 6,755 rough-hairy individuals.

LITERATURE CITED

- (1) HOLLOWELL, E. A., MONTEITH, J., JR., and FLINT, W. P.
1927. LEAFHOPPER INJURY TO CLOVER. *Phytopathology* 17: 399-404, illus.
- (2) JEWETT, H. H.
1932. THE RESISTANCE OF CERTAIN RED CLOVERS AND ALFALFAS TO LEAFHOPPER INJURY. *Ky. Agr. Expt. Sta. Bull.* 329, pp. [157]-172, illus.
- (3) ———
1933. THE RESISTANCE OF LEAVES OF RED CLOVER TO PUNCTURING. *Jour. Econ. Ent.* 26: 1135-1137, illus.
- (4) KOTTUR, G. L.
1922. AN IMPROVED TYPE OF COTTON FOR THE DHARWAR-AMERICAN TRACT. *Agr. Jour. India* 17: 347-352.
- (5) ——— and MARALIHALLI, S. S.
1931. THE USE OF SULPHUR IN THE CONTROL OF RED-LEAF BLIGHT. *Agr. and Livestock in India* 1: 638-641.
- (6) MONTEITH, J., JR., and HOLLOWELL, E. A.
1929. PATHOLOGICAL SYMPTOMS IN LEGUMES CAUSED BY THE POTATO LEAF HOPPER. *Jour. Agr. Research* 38: 649-677, illus.
- (7) NAGAI, I., and SAITO, S.
1923. LINKED FACTORS IN SOY-BEAN. *Japan. Jour. Bot.* 1: 121-136.
- (8) OWEN, F. V.
1927. INHERITANCE STUDIES IN SOYBEANS. II. GLABROUSNESS, COLOR OF PUBESCENCE, TIME OF MATURITY, AND LINKAGE RELATIONS. *Genetics* 12: [519]-529.
- (9) PIETERS, A. J.
1929. RED CLOVER'S HAIRINESS IN AMERICAN TYPES IS DUE TO THE LEAF HOPPER. *U. S. Dept. Agr. Yearbook* 1928: 521-524, illus.

-
- (10) POOS, F. W., and SMITH, F. F.
1931. A COMPARISON OF OVIPOSITION AND NYMPHAL DEVELOPMENT OF
EMPOASCA FABAE (HARRIS) ON DIFFERENT HOST PLANTS. Jour.
Econ. Ent. 24: 361-371, illus.
- (11) SMITH, F. F., and POOS, F. W.
1931. THE FEEDING HABITS OF SOME LEAF HOPPERS OF THE GENUS
EMPOASCA. Jour. Agr. Research 43: 267-285, illus.
- (12) STEWART, R. T., and WENTZ, J. B.
1926. A RECESSIVE GLABROUS CHARACTER IN SOYBEANS. Jour. Amer.
Soc. Agron. 18: 997-1009, illus.
- (13) VEATCH, C.
1930. VIGOR IN SOYBEANS IN RELATION TO INHIBITION OF PUBESCENCE.
Jour. Amer. Soc. Agron. 22: 446-452.
- (14) WOODWORTH, C. M.
1932. GENETICS AND BREEDING IN THE IMPROVEMENT OF THE SOYBEAN.
Ill. Agr. Expt. Sta. Bull. 384, pp. 207-404, illus.
- (15) ——— and VEATCH, C.
1929. INHERITANCE OF PUBESCENCE IN SOY BEANS AND ITS RELATION TO
POD COLOR. Genetics 14: [512]-518.

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No. 5

THE RELATIVE PROTEIN EFFICIENCY AND THE RELATIVE VITAMIN G CONTENT OF COMMON PROTEIN SUPPLEMENTS USED IN POULTRY RATIONS¹

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INTRODUCTION

Considerable evidence has accumulated which indicates that a wide variation exists in the growth-promoting properties of the protein-rich feedstuffs used in poultry rations. These discrepancies have been ascribed largely to differences in quality of proteins. Norris and Heuser (12)³ suggested, however, that these properties may be due not only to protein quality but also to vitamin G content. Later Norris and his coworkers (13) and Bethke, Record, and Kennard (1) showed that the need of poultry for vitamin G is very great and that cereals and their byproducts do not contain sufficient of this vitamin to satisfy the need. It was evident, then, that much of the vitamin G required by poultry was supplied by means of the protein concentrates. Hence the usual conception of the value of these feedstuffs was only partially correct and a further investigation of this field was necessary.

Preliminary studies with chicks to determine the growth-promoting properties of meat scraps, whale-meat meals, and fishmeals, appeared to confirm the suggestion of Norris and Heuser (12). Accordingly, the subsequent work was conducted in two phases, based upon the theory that the value of protein-rich feedstuffs for feeding poultry was due both to protein quality and to vitamin G content. The first of these studies was limited to the evaluation of the proteins by determining their relative efficiency of utilization for growth processes. The second dealt with the quantitative determination of the vitamin G content of these materials. In a preliminary report of this investigation (25), evidence was presented which supports the initial theory. This has been corroborated recently by Record, Bethke, and Wilder (18), who found that haddock meal frequently contains considerable vitamin G as well as protein of fine quality.

THE RELATIVE EFFICIENCY OF THE PROTEINS

METHOD

Originally it was planned to adapt to chicks the method of Mitchell (10) for determining the biological value of proteins. St. John and his coworkers (20) have since reported on such an adaptation.

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² Acknowledgment is hereby made of the cooperation of the Grange League Federation Exchange, Inc., of Ithaca, N. Y., which made this investigation possible by establishing a temporary investigatorship at Cornell University. Acknowledgment is also made of the courtesy of a large number of firms in supplying the writers with many of the samples studied. Space does not permit that they be listed by name.

³ Reference is made by number (italic) to Literature Cited, p. 398.

However, the lack of a suitable synthetic ration capable of supporting the chicks satisfactorily for even a period of 1 week and the lack of an accurate method for differentiating urinary and fecal nitrogen added such variants to this procedure that the necessary assumptions, in addition to those in the original method, might seriously have impaired the value of the data. Furthermore, the practical application of determinations of biological value is difficult, since the effects of digestibility have been eliminated. The biological value, therefore, expresses only one phase of protein utilization, that indicating the extent to which the assimilated protein under study meets the amino acid requirement of the animal used. A simple nitrogen balance trial was therefore decided upon since the results obtained would be a measure of the entire natural process rather than any part of it and could be directly applied in practice. A practical cereal basal ration was used in these studies.

TABLE 1.—*Constitution of the casein control ration*

Ingredients	Parts	Nitrogen	Fat	Ash
	Percent	Percent	Percent	Percent
Yellow corn meal.....	33.82	0.511	1.57	0.52
Wheat-flour middlings.....	20.00	.589	.91	.68
Salt.....	1.00	-----	-----	1.00
Cod-liver oil.....	.50	-----	.50	-----
Liver-extract (dry basis).....	.56	.045	-----	.06
Subtotal.....	55.88	1.145	2.98	2.26
Casein.....	7.69	1.065	.01	.17
Lard.....	1.51	-----	1.51	-----
Bone ash.....	3.57	-----	-----	3.57
Subtotal.....	68.65	2.200	4.50	6.00
Sugar.....	31.35	-----	-----	-----
Total.....	100.00	2.200	4.50	6.00

The casein control ration and the method of standardizing the nitrogen, fat, and ash at the required levels are given in table 1. The amount of wheat-flour middlings was standardized at 20 percent but the corn meal varied in different lots of the basal diet according to the amount necessary to make the total nitrogen from these sources 1.10 percent. The only variation between the rations within a trial was in the protein supplement used and in the amounts of bone ash, lard, and sugar necessary to make the proper adjustments. The total amount of nitrogen supplied was equivalent to 13.75 percent of protein ($N \times 6.25$), which is below the optimum requirement of White Leghorn chicks during the early stages of growth (4).

The liver extract was obtained by a method suggested by R. M. Bethke, of the Ohio Agricultural Experiment Station. Pork liver was ground, cooked in double boilers, dried, reground, and extracted with ether. The residue from 1 kg of dried liver was extracted five times with 2 l of 20-percent alcohol by weight. The combined filtrates were heated to boiling, filtered, and concentrated in vacuo so that 1 ml of extract was equivalent to 1 g of the original dried liver. Assays showed that this should be used at a level of 2 ml, or 0.56 g on the dry basis, per 100 g of ration in order to supply sufficient vitamin G.

This ration was mixed together with the exception of the cod-liver oil and was granulated by moistening, drying at low temperature, and

grinding to pass a 10-mesh screen. By granulation, the feed wastage was reduced to negligible proportions.

The trials were conducted with normal White Leghorn chicks during the sixth and seventh weeks of age. Chicks of this age are still in a period of rapid growth, but are not in a period of active molt. The excreta, therefore, consisted of feces, urine, and slough of skin and feathers. During the first 5 weeks, the chicks were reared on a complete ration containing an excess of the vitamin G complex. They were then distributed evenly according to weight and sex in individual cages, equipped with feeders especially designed to reduce feed wastage to a minimum (fig. 1). Six chicks per lot were used in these studies since preliminary work had indicated that this number was sufficient for consistent results. The room used for this work was well ventilated and was heated with steam thermostatically regulated to maintain the temperature at approximately 70° F. A time clock was used to insure a 15-hour day.

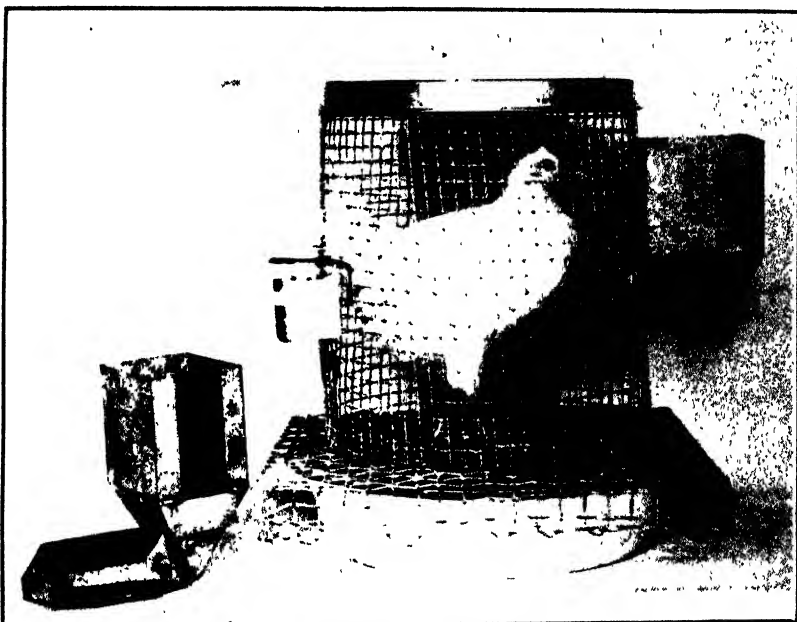


FIGURE 1.—Individual cages, equipped with feeders especially designed to reduce feed wastage to a minimum.

The birds were fed the growth ration for 2 days to accustom them to the cages. They were then given the experimental ration for a preliminary period of 5 days in order to adjust them to the ration and to cleanse the body of residual nitrogen from the growth ration. Preliminary trials had shown that 5 days was adequate for this purpose. During this time the birds were weighed daily at 9 a. m. and were then fed one-half of their daily ration. The remainder was fed at 5 p. m. The amount of feed given each chick daily was equal to 10 percent of the body weight. By this system of feeding the appetites of all chicks, regardless of growth rate, were approximately equally satisfied. This level of feeding was slightly suboptimal.

The experimental period began on the morning of the eighth day and ended at the same time on the fourteenth day. Preliminary studies had shown 6 days to be the optimum length of time. The birds were weighed and were fed as already described except that distilled water was given in place of tap water. Clean cage floors and excreta pans were substituted for those used in the preliminary period as soon as possible after the morning feeding of the eighth day, and the exact hour was noted. In order to prevent loss of ammonia, about 200 ml of distilled water and 10 ml of sulphuric acid were placed in each pan. Several blank pans were likewise treated in order to check the presence of ammonia in the air from outside sources. The birds were removed from the cages on the fourteenth day at the same hour that the excreta pans had been placed in use 6 days previously.

As a general rule, the average gain per bird during this period was about 70 g on the casein control diet. This gradually decreased to about 35 g on diets with a relative protein efficiency of approximately 60. The occasional birds which failed to gain in the same manner as the others in their particular lot or to eat the required amount of feed, or which wasted an undue quantity of feed were eliminated.

The material adhering to the wire-mesh floors was removed into the excreta pans by scrubbing, and the excreta of all birds in each lot left in the experiment were mixed by sex. Concentrated sulphuric acid was poured into each jar of excreta, with constant stirring, to the extent of about 30 ml per bird represented, and the jars were subjected to a temperature of about 60° C. for several days in order that the excreta might undergo partial hydrolysis. The excreta were then rinsed into a tared mixing can, weighed, whipped at high speed in order to thoroughly break up and mix the material, and a sample was weighed out for nitrogen analysis.

The percentage of nitrogen utilized for growth by each sex in each lot was computed from the analyses of the feed consumed and of the excreta, correction being made for any nitrogen found in the blank pans. The percentage storage was then calculated for each sex and the results were averaged by lot. The necessity for doing this was later confirmed by a statistical study by Fisher's method (3, *pp.* 105-107) for paired data of the results of 38 lots on the casein control ration. The average percentage storage for the males was 41.93, and for the females, 40.85. The odds were 49:1 that this difference was not due to chance alone. The relative protein efficiency was determined by dividing the percentage of nitrogen stored from the protein under study by the percentage stored from the casein control lot for that particular trial and multiplying by 100.

TABLE 2.—Consistency of the results obtained in determining relative protein efficiency of fishmeals

Fishmeal no.	Trial no.	Protein storage		Relative efficiency		Fishmeal no.	Trial no.	Protein storage		Relative efficiency	
		Sample	Casein	Sample	Average			Sample	Casein	Sample	Average
		<i>Percent</i>	<i>Percent</i>					<i>Percent</i>	<i>Percent</i>		
12	22	45.2	43.2	105	105	31	32	41.8	43.0	97	96
	33	40.7	39.0	104			37	38.2	39.0	98	
13	22	40.4	43.2	94	94		42	41.8	44.4	94	105
	33	36.6	39.0	94		32	32	44.3	43.0	103	
23	33	37.1	39.0	95	95	32	37	41.9	39.0	107	100
	37	37.0	39.0	95			42	46.8	44.4	105	
30	33	42.7	39.0	109	107	51	39	41.9	41.9	100	106
	37 A	38.4	36.6	105			42	44.5	44.4	100	
	42	47.6	44.4	107		52	39	44.2	41.9	105	
							42	47.2	44.4	106	

Some typical results on fishmeals in which repetitions have been conducted are presented in table 2. These data show that the percentage storage of a particular sample varied somewhat with repetition. An accumulation of such data over a period of several years indicated that this variation was seasonal, tending to be high in the spring and summer and low in midwinter. On the other hand, the results show that this variation was compensated by including the casein pen as a control lot and by expressing the results as relative protein efficiency, since the widest variation in any sample was 4. By using analysis of variance according to Snedecor (22, pp. 13-20), the ratio (F) of the mean square between the means of the fishmeals and the mean square within fishmeals was 33.2. Since a value for F of 5.06 under these conditions is highly significant, the chance of getting a difference greater than 4 in the relative protein efficiency by repetition of a determination was less than 1 in 100. This showed that a difference greater than 4 in relative protein efficiency is probably significant, or very nearly so.

Since the standards for fat and bone ash originally set were exceeded slightly from time to time, the effect of variations in these nutrients and, in addition, the effects of adding moderate amounts of fiber and of varying the protein level were studied. The results, shown in table 3, demonstrated that a considerable deviation was permissible in fat, ash, and protein. The addition of fiber in excess of 0.5 percent, however, materially reduced the efficiency of utilization of protein. These data also showed that the level of nitrogen adopted was well under the optimum requirements for birds at this age on restricted feeding despite the fair rate of growth attained.

TABLE 3.—Effect upon relative protein efficiency of variations in the amounts of various nutrients in the control diet when fed chicks in different pens

Pen no.	Nutrient in diet				Relative protein efficiency	Pen no.	Nutrient in diet				Relative protein efficiency
	Total nitrogen	Total fat	Total ash	Added fiber			Total nitrogen	Total fat	Total ash	Added fiber	
	Percent	Percent	Percent	Percent			Percent	Percent	Percent	Percent	
1.....	2.20	4.5	6	0	100	6.....	2.20	4.5	6	.5	101
2.....	2.20	4.5	8	0	99	7.....	2.20	4.5	6	1.0	97
3.....	2.20	6.0	6	0	97	8.....	2.20	4.5	6	2.0	91
4.....	2.20	7.0	6	0	97	9.....	1.92	4.5	6	0	98
5.....	2.20	7.0	8	0	97	10.....	2.48	4.5	6	0	97

TABLE 4.—Relative protein efficiency of common protein supplements

Protein supplement	Relative protein efficiency of sample—												Average
	a	b	c	d	e	f	g	h	i	j	k	l	
Casein.....													100
Dried skim milk.....	100	101											100
White fishmeal:													
Vacuum dried.....	99	100	100	102	102	105	105	106	106	107	107	111	104
Steam dried.....	103	105											104
Flame dried.....	94	95											94
Sardine fishmeal:													
Domestic.....	94	102											98
Asiatic.....	89	93											91
Menhaden fishmeal:													
Steam dried.....	87	88	92	96									91
Flame dried.....	79	81	95	105									80
Soybean meal:													
Expeller process.....	85	93											89
Hydraulic process.....	76	85											85
Meat scrap:													
75 percent protein.....	67	71											69
60 percent protein.....	71	80											75
55 percent protein.....	73	75	78	78	81	82	83	84	86	86	88	91	82
50 percent protein.....	67	70	71	72	72	73	75	76	78	78			73
45 percent protein.....	72												72
Wheat-meal meal:													
70-75 percent protein.....	73	73											73
55-60 percent protein.....	54	53											53
Corn-gluten meal.....	61												61
Ground soybeans.....	57	60											58

¹ These trials were made with samples produced under experimental conditions.

² These trials were not included in the average as the samples were not representative of general commercial production.

EXPERIMENTAL RESULTS

The results obtained on the protein supplements studied are presented in table 4. Dried skim milk was found to possess the same protein efficiency as casein. It appears, therefore, that the cystine deficiency of casein was adequately overcome by the cereal base used.

The proteins of the whitefish meals prepared by vacuum drying surpassed milk proteins. This was surprising in view of the fact that this product is made from the scrap of the cod and haddock fisheries, which consists chiefly of backs and heads. The meals dried by steam under vacuum possessed no advantage over those dried by steam alone, but they were significantly better than the flame-dried ones. This is in accord with the results of Daniel and McCollum (2), Maynard and his coworkers (7, 8), and Schneider (21). The variation within the vacuum-dried meals was wide. In more detailed studies

of some of these samples, reported elsewhere by the writers (24), this variation was found to be due largely to differences in the method of manufacture.

The domestic sardine meals from the Pacific coast were of high protein efficiency. This meal is wet-rendered and then dried in indirect flame or special steam driers which do not burn the meal when properly operated. Since the inclusion of the "stick" (water-soluble materials) had been found to improve the value of whitefish meals (24), a sample of concentrated stick obtained in the production of one of the sardine meals was added back to the meal in the correct proportion, with the following results:

Protein efficiency of sardine fishmeal — stick	94
Protein efficiency of sardine fishmeal + stick	89

This drop in value was contrary to results obtained on whitefish meals, but appeared significant since these figures were averages of two trials. The Asiatic sardine meals proved to be slightly inferior to the domestic product.

The results obtained on menhaden fishmeal showed that the samples studied were in general somewhat inferior to the other kinds of fish-meals. Maynard, Bender, and McCay (7) and Schneider (21) have also noted the inferiority of this product to whitefish meal. Menhaden meal is produced by wet-rendering and is dried generally in direct flame driers, which may cause some charring unless carefully operated. Some producers are now using steam driers. The menhaden meals produced by flame-drying, with two exceptions, were poorer than those produced by steam-drying. One of these better flame-dried meals was a commercial sample which was comparable in appearance and odor to a good steam-dried product. The other was especially prepared for experimental purposes.

A number of these menhaden fishmeals were produced in pairs from comparable raw ingredients by the various methods of drying. The results obtained are as follows:

Protein efficiency of menhaden fishmeal, vacuum-dried	89
Protein efficiency of menhaden fishmeal, flame-dried	81, 108, and 79
Protein efficiency of menhaden fishmeal, steam-dried	96 and 88

The first pair showed a considerable difference in favor of the special vacuum-dried product over the flame-dried one. These data are in accord with those of Maynard and Tunison (8) obtained with the rat on the same samples. The second pair of samples was produced in a commercial plant, but the flame drier was started up especially for this purpose, and a small batch was put through at a relatively low temperature and in a shorter time than usual. The results were materially in favor of flame-drying under these conditions. The third pair of samples was from the same plant and was prepared with both driers in commercial production. In this case the results were in favor of steam-drying and substantiate those of Maynard and his coworkers (7, 8) obtained with the rat, and those of Schneider (21) obtained with the rat and pig. From these results, it is evident that good menhaden fishmeal can be produced in the direct flame drier only by careful operation.

The soybean meals studied proved to be of slightly lower value than the menhaden fishmeals. The results indicated that meals

produced by the hydraulic process may be as good as expeller meals. The one sample of hydraulic meal that was low had a raw beany flavor, indicating insufficient cooking. The results of Osborne and Mendel (15) showed that soybeans must be cooked thoroughly to obtain the best growth in rats. Robison (19), using swine, found that not only the soybean but also the soybean meal must be subjected to sufficient heat if satisfactory growth is to be obtained. Further unpublished work by the writers substantiates these results.

Variable but somewhat inferior results were obtained from meat scrap. These samples originated from both rendering plants and large packing houses and were all dry-rendered in steam-jacketed melters. Kraybill (6) has described the processes usually followed. This study was restricted mainly to the 50- and 55-percent protein grades, since these represent the bulk of the total production. Despite a wide variation within each grade, there appeared to be a distinct difference in favor of the 55-percent product. The variations in protein efficiency were not found to be correlated necessarily with the bone-ash content of the scraps. Therefore the inferior protein value of 45- and 50-percent protein meat scrap cannot be entirely explained on the basis of higher content of bone proteins. The low efficiency found for the 75 percent protein product is in agreement with the results of Prange, Hauge, and Carrick (17). These samples were prepared from pork cracklings, which Hoagland and Snider (5) have shown to be a poor source of protein for the growth of rats.

The rendering process apparently causes little variation in the protein efficiency of meat scrap, as is shown by the following samples from table 4 which were prepared under controlled conditions:

	50 percent protein grade	55 percent protein grade
Protein efficiency of meat scrap, regular rendered	78	86
Protein efficiency of meat scrap, vacuum rendered.	78	81

The two grades were produced by different packers under commercial conditions. Each pair came from comparable raw ingredients, one being rendered in the regular manner and the other with some vacuum. No beneficial effect was noted from the use of vacuum, since in the first pair the temperature used with the vacuum was so low as to greatly increase the time of processing, and in the second pair the temperature and the length of application were about the same as in the regular method. Theoretically there would be no benefits from the use of vacuum unless either the temperature or the time of processing or both were reduced under the usual procedure. This has been substantiated to some extent by results obtained on several pairs of samples from a third company, as follows:

	50 percent protein grade	55 percent protein grade
Protein efficiency of meat scrap, regular rendered.	67	70
Protein efficiency of meat scrap, vacuum rendered.	72	71

These meat scraps were prepared from comparable raw material, apparently of lower inherent value than those just discussed. The vacuum process was a commercial one in which a high vacuum was used, and the time and temperature of processing were both less than in the regular method. The results were slightly in favor of vacuum-

rendering. It is probable, therefore, that the variations in the protein quality of meat scraps are caused largely by differences in the raw materials used, as concluded by Pope (16).

Whale-meat meal on the whole proved to be of low protein efficiency, although the two high protein samples ranked with 50-percent meat scrap. No definite information was available as to the method of production of these samples. The two higher grade meals were of South American and South African origin, the other two samples were from the South Georgia Islands.

The low protein efficiency obtained on the samples of soybeans is in agreement with the results of other workers, who found that raw soybeans were an unsatisfactory source of protein for the growth of rats (Osborne and Mendel (15)), pigs (Robison (19)), and chicks (Tomhave and Mumford (23)).

Corn-gluten meal was of low protein efficiency in the basal ration used, but in further work conducted at this station, it has been found possible to supplement corn-gluten meal so as to make it satisfactory.

One sample each of steam-dried blood meal and of vacuum-dried blood flour were included in these studies, but these products rendered the rations so unpalatable that food consumption was barely sufficient for maintenance. Hence, these results are not given. Further work on a practical diet showed that either product could be used to replace meat scrap to the extent of not over 2 percent of the total mash mixture without materially affecting protein efficiency. Hoagland and Snider (5), using the rat, and Winter (26), using swine, have also found dried blood to be of low protein value and unpalatable.

Sufficient evidence has been accumulated to demonstrate the variations in relative protein efficiency among and within the common protein supplements studied, although the number of samples in some cases was limited. The comparative value of the various classes of protein supplements is in general agreement with the results which Mussehl and Ackerson (11) obtained on single samples of some of these materials, using the growth of chicks as a criterion. The variations encountered could be explained not only by inherent differences in the raw materials but also by differences in the processes of manufacture used in producing the finished products. In order to compensate for these unavoidable variations in practice, rations should be formulated with a sufficient excess of protein to provide a margin of safety.

THE RELATIVE VITAMIN G CONTENT

In the first part of this paper the writers have pointed out that wide variations in growth-promoting properties occurred among and within the common protein supplements used in poultry feeding, and data have been presented to show that at least part of these variations was due to differences in relative protein efficiency. Evidence was presented in a preliminary report by Wilgus, Ringrose, and Norris (25) that these materials likewise varied in quantity of the vitamin G complex and that this also accounted in part for such differences in growth-promoting properties. In order to verify the preliminary results and to determine more accurately the range in the vitamin G potency of such materials, the relative vitamin G content of most of the samples used in the previous report, as well as of some additional ones, was studied.

METHOD

The assays were made on White Leghorn chicks by a method developed at this laboratory. Chicks were grown on a wire floor to 2 weeks of age on a ration deficient in vitamin G. Preliminary work had shown that this period was adequate to deplete chicks of their natural reserve of this factor. They were then distributed equally in all lots according to weight, the extremes being eliminated. Approximately 70 percent of the chicks originally started were used in the experimental trials. In the seven trials reported here, an average of 20 chicks per lot was used, but the number ranged from 15 to 22 in the different trials.

After distribution, the chicks were individually banded, reweighed, and then fed the basal ration, supplemented by 5 or 10 percent of the materials under study. One lot was continued on the basal ration as a negative control. During the experimental period, records of food consumption and of individual weights were made weekly.

The basal ration was composed of wheat-flour middlings, 20; yellow corn meal, 54; cod-liver oil, 1; purified casein⁴ to make 25 percent of protein on the dry basis; bone ash and refined cottonseed oil to standardize the bone ash at 2 percent and the fat at 6 percent in all cases; and cornstarch to make 100 parts. In the supplemented rations, 5 or 10 percent of cornstarch was replaced by the supplement, and the levels of protein, fat, and bone ash were adjusted to the required amounts. In the last two trials, it was necessary to increase the level of bone ash to 3 percent in order to include certain meat scraps high in this respect. No effect of this variation could be noted.

The basal ration used in this investigation was nutritionally complete insofar as known except in the vitamin G complex. It was greatly deficient in the growth-promoting phase of this complex but seldom deficient in the antipellagic phase, and then so slightly that growth was not retarded. On the other hand, the nutritional paralysis associated with this complex (Norris et al. (14); Bethke et al. (1)) did occur to a moderate extent, but it was not severe enough to affect growth results except in a few cases. Such individuals were eliminated.

In four experiments, the birds were continued on the experimental diets for 6 weeks in order to determine the proper length for the experimental period. Six weeks proved to be too long, as the growth rate slowed up toward the end of that time and the need for vitamin G was reduced. As a result, the birds on the higher levels of vitamin G gained at a proportionately lower rate because of the operation of the law of diminishing increment. The net result was an apparent drop in potency, particularly when the growth on the negative control ration was higher than usual on account of variations in the vitamin G content of the basal ration. This effect, however, was not evident

⁴ Commercial casein was purified of vitamin G by dissolving 1 part in 10 parts of dilute sodium hydroxide solution, adjusting the pH to 9.0, and stirring constantly for 16 hours at a temperature of approximately 50° C. The casein was precipitated with hydrochloric acid, drained, washed four times with tap water, and dried.

during the first 4 weeks. The experimental period was limited therefore to 4 weeks.

It was felt that in determining the vitamin G content of protein concentrates the law of diminishing increment might interfere with the interpretation of the results. Hence, a study was made of the effect of this law on feeding graded quantities of vitamin G, dried pork liver being used to supply this vitamin. A straight-line curve

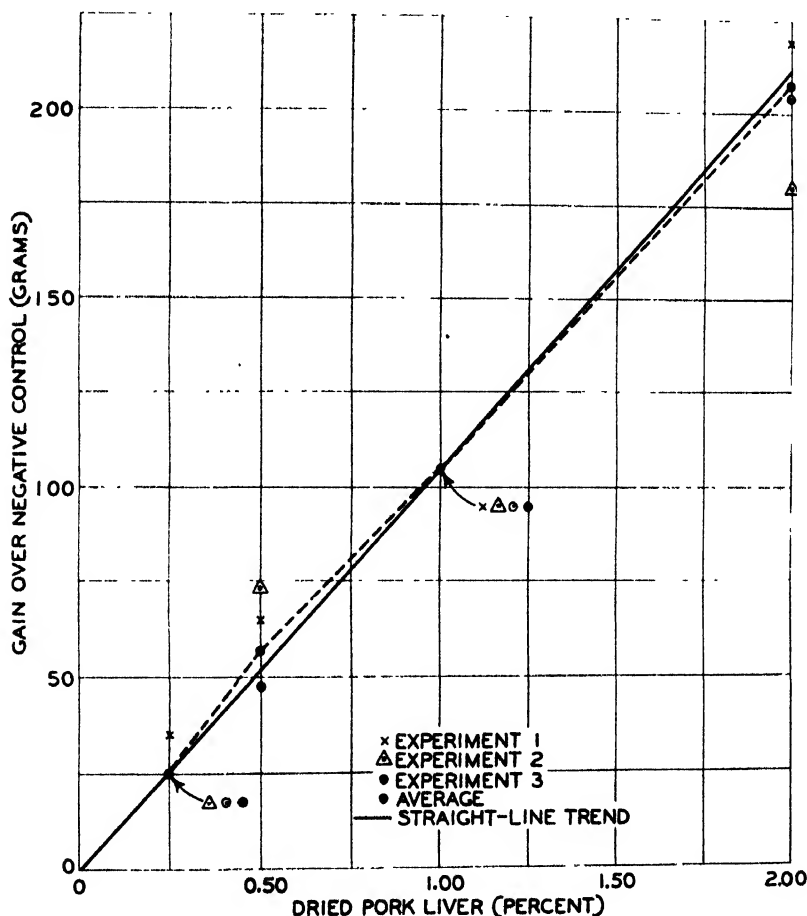


FIGURE 2.—Weight gains of chicks fed dried pork liver as a source of vitamin G over gains of negative control birds.

was found to fit best the data obtained. These data and the straight-line curve are presented in table 5 and figure 2, respectively. This study demonstrated that at the levels of vitamin G used the law of diminishing increment had not become appreciably effective and that, unless unusually large quantities of the protein concentrates were fed, this law would not influence the accuracy of this method of determining the vitamin G content.

TABLE 5.—*Weight gains of chicks fed dried pork liver at different levels as a source of vitamin G over gains of negative control birds*

Dried pork liver fed (percent)	Gain over negative control birds in—							
	Experiment 1		Experiment 2		Experiment 3		Average	
	Gain	Standard error	Gain	Standard error	Gain	Standard error	Gain	Standard error
	<i>Grams</i>		<i>Grams</i>		<i>Grams</i>		<i>Grams</i>	
0.25	34.8	17.69	25.1	11.86	25.0	8.54	26.3	6.45
.50	64.5	12.34	73.2	16.43	47.4	9.93	56.8	6.95
1.00	104.8	19.42	104.8	23.11	105.9	12.81	105.4	9.69
2.00	219.2	8.84	179.8	14.40	203.9	13.64	207.4	6.60

In calculating the data presented in table 5, the mean of the average weight of males and females in each pen and its standard error

were obtained by the approximate formula, $\frac{A+B}{2} \pm \frac{\sqrt{a^2+b^2}}{2}$, derived

from the exact formula given by Mellor (9, p. 553). This was used in order not to give too much weight to the females, which by nature are lighter than males and therefore have a smaller standard error. Three individuals which lost weight or failed to gain, usually presaging death, were eliminated out of a total of 299 birds. However, in averaging the lot gains made in the three trials on the same

level of pork liver, the more exact formula (Mellor (9)), $\frac{\frac{A}{a^2} + \frac{B}{b^2}}{\frac{1}{a^2} + \frac{1}{b^2}}$

$\sqrt{\frac{1}{\frac{1}{a^2} + \frac{1}{b^2}}}$, was employed in order to give the greatest weight to the

average gain with the smallest probable error, thus giving the greater influence to those lots of chicks in which the least deviation occurred and in which larger numbers were used.

The straight-line curve (fig. 2) was used in determining the relative vitamin G potency of the protein-rich feedstuffs studied. At the end of the 4 weeks' experimental period the average gain per lot over the negative control lot was determined and plotted upon this curve to find the quantity of dried pork liver which gave an equivalent gain. Upon dividing this by the percentage of protein concentrate used and multiplying by 100, the potency in the growth-promoting phase of the vitamin G complex was obtained relative to dried pork liver as 100 percent.

Care was taken to use such levels of the protein concentrates that the gains would fall upon the straight-line curve. In cases in which 10 percent of supplement was added, there appeared to be some possibility that if a supplement of low protein quality were used, the protein efficiency of the diet might be low enough to be a limiting factor. However, in no case was the amount of casein used small

enough to reduce the combined proteins of the corn meal, middlings, and casein to less than 18 percent. Since the results obtained with this type of diet by Heuser and Norris (4) indicate that this quantity of protein supports maximum growth, it is improbable that protein efficiency was a limiting factor at any time.

EXPERIMENTAL RESULTS

The relative vitamin G content of the protein supplements used in these experiments is given in table 6. The results in table 5 and those presented in an earlier study (24) show that a difference of about 20 g in the gains over the negative control lot was significant when replicate trials were conducted. By interpolating on the straight-line curve shown in figure 2, a gain of 20 g was found to be equal to that produced by 0.20 percent of dried pork liver; therefore a difference in relative vitamin G content of 4, when 5 percent of the material under study was used, or of 2, when 10 percent was used, was significant. It was concluded, therefore, that as a general rule a difference of 5 or more in relative vitamin G content was significant.

TABLE 6.—Relative vitamin G content of common protein supplements

Protein supplement	Relative vitamin G content of sample —										Average
	a	b	c	d	e	f	g	h	i	j	
Dried pork liver.....											100
Dried skim milk.....	17	17	19	20	20						19
White fishmeal:											
Vacuum-dried.....	15	17	18	8	19	10	112	112	113	117	10
Steam-dried.....	13	16									5
Flame-dried.....	15										5
Sardine fishmeal:											
Domestic.....	9	12	13								9
Asiatic.....	5										5
Menhaden fishmeal:											
Steam-dried.....	2	5	6	8							5
Flame-dried.....	11	5	6								4
Soybean meal:											
Expeller process.....	3	4									4
Hydraulic process.....	3	3									3
Meat scrap:											
75 percent protein.....	6	5									6
60 percent protein.....	5	6	10								7
55 percent protein.....	4	4	5	6	7	7	11	11	14		5
50 percent protein.....	3	3	4	5	6	6	7	11	11	14	6
45 percent protein.....	5	7									6
Corn-gluten meal.....	0										0
Dried blood.....	0	0									0
Ground soybeans.....	2	3	3	4							3

¹ These trials were made with samples produced under experimental conditions.

² These trials were not included in the average as the samples were not representative of general commercial production.

The value found for dried skim milk was about one-fifth that of dried pork liver. The individual samples possessed potencies within the limits of experimental error. These were all samples representative of commercial production, 4 being roller-dried and 1 spray-dried. The high feeding value attributed to dried skim milk in the past appears to be justified, for the samples studied were uniformly high both in relative protein efficiency and in relative vitamin G content. However, this product is probably of primary value as a source of vitamin G, since some protein supplements nearly equal or even sur-

pass it in protein efficiency when combined with the proteins of corn and wheat (24).

The haddock meals possessed about one-half the vitamin G potency of dried skim milk. The vacuum-dried meals were superior to those dried by steam alone or by flame. The variations within the vacuum- and steam-dried samples had previously been shown by the writers (24) to be due largely to the method of manufacture and the types of ingredients used. They also showed that the vitamin G content of this kind of fishmeal when properly prepared was sufficient to be of considerable practical importance. This finding is supported by the results of Record, Bethke, and Wilder (18).

The domestic sardine meal was comparable with whitefish meal in vitamin G content in spite of the fact that this was a wet-rendered flame-dried product. That this product may have an inherent vitamin G content as high as that of whitefish meal is evidenced by the high value found in an experiment where the stick was returned to the wet-rendered meal in the correct proportion. The results obtained were as follows:

Relative vitamin G content of sardine fishmeal—stick	9
Relative vitamin G content of sardine fishmeal+stick	13

The latter value approximated that of dry-rendered vacuum-dried whitefish meals prepared under proper conditions. This fact indicates that the indirect flame drier used may not have had a more destructive effect on the vitamin G content of the meal than the vacuum type used for whitefish meals. The stick of sardine scrap had the same relative potency as that found for haddock meal in which about one-third of the total vitamin G content of the scrap was found in the stick (Wilgus et al. (24)). The Asiatic sardine meal was inferior to the domestic sardine meal in vitamin G content.

The menhaden fishmeals studied were inferior to the other domestic fishmeals. The variations encountered were rather wide and could be only partially explained on the basis of method of manufacture. This was noted in studies of several samples produced in pairs from comparable raw material, the results of which are as follows:

Relative vitamin G content of menhaden fishmeal, steam-dried	5 and 8
Relative vitamin G content of menhaden fishmeal, flame-dried	1 and 6

The difference was in favor of steam-drying, but it was not sufficient to be of practical importance. The values found approximated those of wet-rendered whitefish meals similarly prepared (24), which indicates that this fish scrap may be inherently as high in vitamin G as the other varieties studied. Under present methods of production, however, menhaden fishmeal does not appear to be a very dependable source of vitamin G, although it averages about one-fourth the potency of dried skim milk.

The soybean meals were uniformly low in vitamin G. There was no difference between the meals produced by the hydraulic and the solvent process. These possessed the same vitamin potency as the ground soybeans.

The meat scraps, on the whole, were of about the same potency as the menhaden fishmeals. The averages obtained for the various grades were practically identical although there was a considerable range within each grade. The reasons for these variations appear to lie partly in the ingredients used, as all but three were dry-rendered

by the usual process. This is reasonable to suppose because of the nature of the raw materials. On the other hand, some variation may have been due to differences in the temperature of rendering and length of application, since there appears to be no standard procedure in this regard, each producer varying the size of charge, steam pressure, and time to suit his conditions or prejudices. The effect of such variations was noted in a study of paired samples, each pair produced from comparable raw ingredients. These results are as follows:

Relative vitamin G content of meat scrap, regular-rendered.....	14, 11, 3, 3, and 4
Relative vitamin G content of meat scrap, vacuum-rendered.....	11, 14, 5, 4, and 7

The first two pairs were made from ingredients containing some livers. In these no beneficial effect was found from the use of vacuum since in the first pair the temperature was so low that the length of application necessary was greatly increased, and in the second pair, the temperature and time were essentially the same as in the regular method. On the other hand, a slight difference in favor of a special vacuum method was noted in the last three samples, although the values were low, due probably to the inherent value of the ingredients. In these three cases, the temperature was considerably lower and the length of application considerably shorter than are usual when the regular method is employed. This fact suggests that the nutritive value of meat scrap may be better preserved by using the smallest amount of heat possible to render it properly. However, the fluctuations in potency found for all the meat scraps studied show that they were not very reliable sources of vitamin G, although they averaged about one-third the potency of dried skim milk.

Neither steam- nor vacuum-dried blood meal nor corn-gluten meal was found to contain any vitamin G.

The results of this study show that most of the products assayed contained variable quantities of vitamin G. This, therefore, confirms the original theory that the growth-promoting properties of this class of feedstuffs may be due to vitamin G content as well as to protein quality. This is further substantiated by the results obtained by Record, Bethke, and Wilder (18) on haddock meal. Sufficient samples have been assayed in many cases to indicate the scope of the variations among and within these products. In addition, some samples produced under controlled conditions have demonstrated that these variations may be due not only to the inherent potency of the raw materials but also to the methods of manufacture. The data presented therefore emphasize the importance of using types of raw material of high inherent value, of manufacturing them by methods known to preserve best such value, and of making economic use of the nutritive properties of such products.

SUMMARY

The utilization for growth processes of the protein of protein-rich feedstuffs combined with an equal quantity of protein from yellow corn meal and wheat-flour middlings was determined by means of nitrogen balance trials. Final results were expressed as relative protein efficiency. These values were obtained by dividing the percentage of protein stored from a given ration by normal White Leghorn chicks

during the seventh week of age by that from a standard casein ration, and multiplying by 100.

The relative protein efficiency of a number of the common protein supplements used in poultry rations has been determined. The materials studied, listed in order of their efficiency, are as follows: Vacuum- and steam-dried white fishmeals, 104; dried skim milk, 100; domestic sardine fishmeal, 98; flame-dried white fishmeal, 94; steam-dried menhaden fishmeal and Asiatic sardine meal, 91; soybean meal, 88; flame-dried menhaden fishmeal, 80; meat scrap, 77; whale-meat meal, 64; corn-gluten meal, 61; ground soybeans, 58. These results show that the variations in growth-promoting properties among and within the various classes of common protein supplements studied may be explained at least in part by variations in their relative protein efficiency. These variations were apparently due not only to differences inherent in the raw materials from which these products were obtained, but also to the various factors involved in their manufacture.

A method for determining the relative growth-promoting vitamin G content of feedstuffs has been developed, based upon the gain produced over the control diet by adding 5 or 10 percent of the supplement under study. This was expressed in terms of dried pork liver affording an equivalent gain, and the final results were calculated in percentage with dried pork liver as 100.

A number of samples of common protein supplements used in poultry rations have been assayed by this method. The materials studied, listed in order of their vitamin G potency, are as follows: Dried skim milk, 19; vacuum-dried white fishmeal, 10; domestic sardine fishmeal, 9; meat scrap, 6; steam- and flame-dried white and menhaden fishmeals and Asiatic sardine fishmeal, 5; soybean meal and ground soybeans, 3; corn-gluten meal and dried blood, 0. Thus the growth-promoting properties of the protein concentrates were found in general to be due to vitamin G content as well as to quality of protein. The range within many of the products was found to be rather wide. This was traced to methods of manufacture and types of ingredients used.

LITERATURE CITED

- (1) BETHEKE, R. M., RECORD, P. R., and KENNARD, D. C.
1931. A TYPE OF NUTRITIONAL LEG PARALYSIS AFFECTING CHICKS. *Poultry Sci.* 10: 355-368, illus.
- (2) DANIEL, E. P., and MCCOLLUM, E. V.
1931. STUDIES ON THE NUTRITIVE VALUE OF FISH MEALS. U. S. Dept. Com., Bur. Fisheries Invest. Rept. 2, 19 pp., illus.
- (3) FISHER, R. A.
1930. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London.
- (4) HEUSER, G. F., and NORRIS, L. C.
1934. THE INFLUENCE OF THE PROTEIN LEVEL ON THE GROWTH OF CHICKENS AND ITS RELATION TO SUBSEQUENT BEHAVIOR. 5th Cong. Mondiale di Pollicultura Atti, v. 2, pp. 551-558, illus.
- (5) HOAGLAND, R., and SNIDER, G. G.
1926. THE NUTRITIVE VALUE OF PROTEIN IN BEEF EXTRACT, OX BLOOD, OX PALATES, CALF LUNGS, HOG SNOUTS, AND CRACKLINGS. *Jour. Agr. Research* 33: 829-843, illus.
- (6) KRAYBILL, H. R.
1928. WHAT ARE TANKAGE, MEAT SCRAPS, AND MEAT MEAL. *Poultry Sci.* 8: 11-18.

- (7) MAYNARD, L. A., BENDER, R. C., and McCAY, C. M.
1932. VITAMIN A AND PROTEIN CONTENT OF VARIOUS FISH MEALS. *Jour. Agr. Research* 44: 591-603, illus.
- (8) ——— and TUNISON, A. V.
1932. INFLUENCE OF DRYING TEMPERATURE UPON DIGESTIBILITY AND BIOLOGICAL VALUE OF FISH PROTEINS. *Indus. and Engin. Chem.* 24: 1168-1171.
- (9) MELLOR, J. W.
1929. HIGHER MATHEMATICS FOR STUDENTS OF CHEMISTRY AND PHYSICS, WITH SPECIAL REFERENCES TO PRACTICAL WORK. New impression, 641 pp., illus. London, New York [etc.].
- (10) MITCHELL, H. H.
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. *Jour. Biol. Chem.* 58: 873-903.
- (11) MUSSEHL, F. E., and ACKERSON, C. W.
1931. UTILIZATION OF PROTEINS BY THE GROWING CHICK. *Nebr. Agr. Expt. Sta. Research Bull.* 55, 19 pp., illus.
- (12) NORRIS, L. C., and HEUSER, G. F.
1930. THE RELATION OF THE PROTEIN REQUIREMENT OF CHICKS TO THE RATE OF GROWTH. I. THE QUANTITY OF PROTEIN REQUIRED BY CHICKS DURING EARLY GROWTH. *Poultry Sci.* 9: 378-392, illus.
- (13) ——— HEUSER, G. F., RINGROSE, A. T., WILGUS, H. S., JR., and HEIMAN, V.
1934. THE VITAMIN G REQUIREMENT OF POULTRY. 5th Cong. Mondiale di Pollicultura Atti, v. 2, pp. 512-520.
- (14) ——— HEUSER, G. F., WILGUS, H. S., JR., and RINGROSE, A. T.
1931. THE OCCURRENCE IN CHICKS OF A PARALYSIS OF NUTRITIVE ORIGIN. *Poultry Sci.* 10: 93-97, illus.
- (15) OSBORNE, T. B., and MENDEL, L. B.
1917. THE USE OF SOY BEAN AS FOOD. *Jour. Biol. Chem.* 32: 369-387, illus.
- (16) POPE, E. A.
1929. REPORT ON THE NUTRITIVE VALUES OF MEAT-MEALS. New Zeal. Dept. Sci. and Indus. Research Bull. 12, 27 pp., illus.
- (17) PRANGE, R. W., HAUGE, S. M., and CARRICK, C. W.
1927. THE STUDY OF THE PROTEIN IN A COMMERCIAL MEAT PRODUCT. *Poultry Sci.* 6: 302-307, illus.
- (18) RECORD, P. R., BETHKE, R. M., and WILDER, O. H. M.
1934. EFFECT OF METHOD OF MANUFACTURE ON THE NUTRITIVE VALUE OF FISHMEALS AS DETERMINED BY GROWTH STUDIES WITH CHICKS. *Jour. Agr. Research* 49: 715-722.
- (19) ROBISON, W. L.
1930. SOYBEANS AND SOYBEAN OILMEAL FOR PIGS. *Ohio Agr. Expt. Sta. Bull.* 452, 42 pp., illus.
- (20) ST. JOHN, J. L., JOHNSON, O., CARVER, J. S., and MOORE, S. A.
1932. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN IN THE STUDY OF AVIAN NUTRITION. *Jour. Nutrition* 5: 267-276.
- (21) SCHNEIDER, B. H.
1932. NITROGEN-BALANCE STUDIES WITH VARIOUS FISH MEALS. *Jour. Agr. Research* 44: 723-732.
- (22) SNEDECOR, G. W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp., illus. A. res. (Iowa State Col. Agr. and Mech. Arts, Div. Indus. Sci. Monog. 1.)
- (23) TOMHAVE, A. E., and MUMFORD, C. W.
1933. GROUND SOYBEANS AS A PROTEIN SUPPLEMENT FOR GROWING CHICKS. *Del. Agr. Expt. Sta. Bull.* 183, 24 pp., illus.
- (24) WILGUS, H. S., JR., NORRIS, L. C., and HEUSER, G. F.
1935. THE EFFECT OF MANUFACTURING PROCESS UPON THE NUTRITIVE VALUES OF HADDOCK MEAL. *Indus. and Engin. Chem.* 27: 419-422.
- (25) ——— RINGROSE, R. C., and NORRIS, L. C.
1934. STUDIES OF THE ESSENTIAL NUTRITIVE PROPERTIES OF COMMON PROTEIN SUPPLEMENTS USED IN POULTRY RATIONS. 5th Cong. Mondiale di Pollicultura Atti, v. 2, pp. 541-548.
- (26) WINTER, A. R.
1929. THE NUTRITIVE VALUE OF BLOOD-MEAL PROTEIN FOR GROWTH. *Ohio Agr. Expt. Sta. Bull.* 436, 42 pp.

THE NUTRITIVE VALUE OF THE PROTEINS OF CORN-GLUTEN MEAL, LINSEED MEAL, AND SOYBEAN-OIL MEAL¹

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INTRODUCTION

The results of previous experiments from this laboratory (22)² indicated no differences in the utilization by growing lambs of the proteins of alfalfa hay and clover hay when fed at a 10-percent level in rations which were presumably adequate in total digestible nutrients, minerals, and vitamins. Furthermore, no differences were observed between the biological value of the proteins of an alfalfa and corn ration and the proteins of a clover and corn ration when both rations were fed at a 10-percent level of protein. The biological values of the proteins of the two latter rations were approximately the same as when each of the hays formed the only source of protein, hence showing no supplementary effect due to the addition of corn protein.

The average of the biological values was 81 for clover protein, 79 for alfalfa protein, 80 for the protein in the combination of clover and corn, and 77 for the protein in the combination of alfalfa and corn. These results were interpreted to indicate that alfalfa hay and clover hay are probably not deficient in quality of protein for sheep when fed in a balanced ration as regards protein and total digestible nutrients.

Since there were no significant differences in protein utilization in these experiments, the question was raised in the minds of the writers as to whether or not there are any actual differences in the nutritive value of the proteins of common feeds for sheep, when the feeds in question are fed in rations which furnish sufficient of total digestible nutrients and other known dietary essentials, and when fed at the same level of protein intake. Very few data have been presented which show any marked differences in the efficiency of protein utilization when common feedstuffs have been fed to ruminants in well-balanced rations (5, 6, 7, 11, 19). The common explanation given is that the ruminant has the ability to synthesize certain of the essential amino acids as a result of bacterial action in the digestive tract. This possibility has been frequently pointed out in the literature, especially with reference to the synthesis of cystine by sheep.

These experimental results (22) have suggested the desirability of obtaining further data on the importance of the quality of protein for ruminants and of ascertaining whether differences in the biological value of proteins can be obtained with the sheep using the nitrogen-balance type of experimentation. Therefore, experiments have been conducted to determine the nutritive values for sheep of the proteins

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² Reference is made by number (italic) to Literature Cited, p. 411.

in three common protein-rich feeds which, on the basis of origin and general nutritive value, should furnish proteins differing in quality.

MATERIALS

Soybean-oil meal was selected as one of the few plant protein supplements that furnish proteins of high quality. Though some experiments have indicated that soybean proteins are rather low in cystine (17, 21), they apparently supply more adequate amounts of the essential amino acids than most single seeds or seed byproducts.

In experiments with swine or rats the proteins of soybean-oil meal would probably rank above those of linseed meal and corn-gluten meal. Linseed meal is a popular protein concentrate, but some experiments have shown that its protein is not of the highest quality (10, 15, 19). Corn proteins, especially those of the endosperm, are low in two of the essential amino acids—tryptophane and lysine. Corn-gluten meal is probably even lower than the entire corn grain in tryptophane and lysine, as it commonly includes none of the germ proteins of which are of better quality than those of the endosperm. The immediate object of this investigation, therefore, was to determine what differences, if any, would be found in the nutritive value of the proteins of soybean meal, linseed meal, and corn-gluten meal for growing lambs.

EXPERIMENTAL PROCEDURE

In experiments with swine or rats the proteins of soybean-oil meal tuted for part of the starch and sugar in a low-nitrogen ration and fed to growing wether lambs. The nitrogen balances were determined on each ration, and also the digestibility of the protein, the storage of protein, and the biological value of the proteins (12, 13).

The percentage composition of feeds used in this experiment is shown in table 1.

TABLE 1.—Percentage composition of feeds used

Feed	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
Corn-gluten meal.....	93.95	2.80	42.36	1.68	4.96	42.15
Linseed meal.....	93.35	5.11	39.01	5.56	7.44	36.23
Soybean-oil meal.....	93.05	5.06	42.60	5.73	5.72	33.34

The soybean-oil meal was an expeller-process product and had a pleasant cooked odor and nutlike taste. All the feeds were apparently of good quality and were fed as obtained from the feed manufacturer.

Each of the feeds was added to a low-nitrogen ration of ground wheat straw, cellulose, cornstarch, sugar, corn oil, and salt mixture in such amounts as to provide a protein level of about 11 percent. Of this 11 percent protein, 10 percent came from the feed in question and 1 percent from the straw and other ingredients. The same amount of straw was included in all the rations to furnish bulk, and different amounts of pure cellulose³ were included to equalize the concentration of crude fiber. Raw cornstarch, cane sugar, and corn oil were included in order to increase and equalize the total energy

³ Washed sylphrap.

content of the rations. The composition of the rations is given in table 2.

TABLE 2.—Percentage composition of experimental rations

Constituent	Low-nitrogen ration	Corn-gluten-meal ration	Linseed-meal ration	Soybean-oil-meal ration
Corn-gluten meal		23.6		
Linseed meal			25.7	
Soybean-oil meal				23.5
Straw, wheat	25.0	25.0	25.0	25.0
Cellulose, regenerated	10.5	9.7	9.0	9.5
Starch	28.0	17.4	17.2	18.0
Sugar	28.0	17.3	17.1	18.0
Corn oil	4.5	4.0	3.0	3.0
Salt mixture	4.0	3.0	3.0	3.0
Total	100	100	100	100
Protein content (N×6.25) ¹	1.15	10.99	11.0	10.96

¹ These percentages of nitrogen represent the averages of analyses on 3 mixes of the low-nitrogen ration and 6 mixes of each of the other 3 rations.

The salt mixture was similar in composition to the one designed by Woodward and McCay (23) which is used in this laboratory in synthetic diets for herbivorous animals. The lambs were allowed free access to common salt during all preliminary and intervening periods. A vitamin A and D concentrate was added during experiment 2. It was fed at the rate of 0.0125 g per kilogram of live weight per day. No vitamin B supplements were added because this would have involved adding nitrogen to the ration. The importance of and necessity for vitamins in short balance experiments of this type do not seem to be definitely known.

Three young growing wethers were used. They were purebred Shropshire lambs from the university flock. The details involved in carrying out the experiments and the methods of analysis used were the same as those employed in the previous experiments (22). The experimental periods were 10 days in length. The preliminary and intervening periods were also 10 days in length, except those preceding the low-nitrogen periods, which were generally longer.

The lambs were fed twice a day. The plan followed in feeding each lamb on the different rations is shown in table 3:

TABLE 3.—Plan followed in feeding each lamb the different rations in experiments 1 and 2

EXPERIMENT 1

Lamb no. 6	Lamb no. 7	Lamb no. 8
Low nitrogen	Low nitrogen	Low nitrogen.
Corn-gluten meal	Linseed meal	Soybean-oil meal.
Linseed meal	Soybean-oil meal	Corn-gluten meal.
Soybean-oil meal	Corn-gluten meal	Linseed meal.
Low nitrogen	Low nitrogen	Low nitrogen.

EXPERIMENT 2

Low nitrogen	Low nitrogen	Low nitrogen.
Soybean-oil meal	Corn-gluten meal	Linseed meal.
Linseed meal	Soybean-oil meal	Corn-gluten meal.
Corn-gluten meal	Linseed meal	Soybean-oil meal.
Low nitrogen	Low nitrogen	Low nitrogen.

The same low-nitrogen period was used for the last period of experiment 1 and for the first period of experiment 2. The second experiment was, therefore, a repetition of experiment 1 with the reversed order of feeding the different rations to each lamb.

DISCUSSION OF RESULTS

The complete results of the metabolism experiments are presented in tables 4 and 5. For convenience of study, the final values are summarized in table 6.

Considerable variations in individual values are noted in a few cases, but they are no greater than commonly occur in experiments of this type. The average results for each of the two trials agreed quite closely in almost all respects. Further repetition of this work and experiments with other species would be desirable.

There was little difference in the average digestibility of the proteins of the corn-gluten-meal and the soybean-oil-meal rations. The proteins of both these rations, however, were slightly more digestible than the proteins of the linseed-meal ration.

There were some differences in the amounts of nitrogen retained or stored on the different rations. The storage from the soybean-oil-meal ration was significantly higher than from either of the other two rations. The average storage of nitrogen was 33.8 percent for the soybean-meal ration, 26.7 percent for the linseed-meal ration, and 26.5 percent for the corn-gluten-meal ration. The odds (9) are 302 to 1 against the difference in protein storage between soybean-oil meal and linseed meal being due to chance alone. For the other comparison, the odds were 32 to 1 in favor of soybean-oil meal as compared with corn-gluten meal. There were similar differences in the percentages of digested nitrogen stored. The average percentages of digested nitrogen stored were 51 for soybean-oil meal, 39.8 for corn-gluten meal, and 41.5 for linseed meal. These results clearly show a superiority of the proteins furnished by soybean-oil meal.

The biological values of the protein in the soybean-oil-meal ration were slightly higher than those for either corn-gluten meal or linseed meal. The average was 72.8 percent for soybean-oil-meal proteins, 65.7 for the corn-gluten meal, and 67.7 percent for linseed-meal proteins. The difference between the biological values of soybean meal and of linseed meal is significant as shown by odds of 87 to 1 against this difference being due to chance. A tendency toward statistical significance is also shown in comparing the soybean-oil-meal ration with the corn-gluten-meal ration. The odds in this case are 20 to 1 in favor of the soybean-oil-meal ration.

The proteins of linseed meal were less digestible than corn-gluten-meal proteins but they were utilized a little more efficiently on the average. Soybean proteins were digested at approximately the same rate as the corn-gluten-meal proteins, but were more efficiently utilized as indicated by the storage and biological values of the proteins. These results, as a whole, indicate that soybean-oil meal furnishes a more efficient combination of amino acids than does either linseed meal or corn-gluten meal.

The data on digested protein (nitrogen) stored show greater differences in favor of the soybean-oil-meal proteins than do the biological values. When the metabolic and endogenous nitrogen losses are

considered, and the biological values of the protein computed, the differences in the average values between the soybean-oil-meal ration, and the other two rations becomes less. Since the two methods of evaluating the proteins differ only in the respect that metabolic and endogenous nitrogen are considered in the calculation of biological values, these data may question the accuracy of estimating these nitrogen losses. The writers recognize that the low-nitrogen ration used is not entirely satisfactory for sheep and for most other species. With prolonged feeding of the low-nitrogen ration there is a diminished appetite and falling off in food consumption. The data from the low-nitrogen feeding periods show that as the feed consumption decreases the rate of metabolic nitrogen excreted per unit of feed intake will increase, thus producing an error in the calculation of the biological values. This error, however, may not be very great.

Reduced feed consumption during a period of low-nitrogen feeding will leave an animal in an abnormal condition and may possibly alter the reliability of the results in the following experimental periods. These experiments were conducted in such a manner that each ration was fed to two lambs just following a low-nitrogen period and then again just preceding a low-nitrogen period. Without a single exception, the data show that higher biological values for each feed were obtained immediately following the periods of low-nitrogen feeding. These observations are not in agreement with those of Mitchell (14) who believes that a period of low-nitrogen feeding will exert no appreciable effects on the utilization of protein in subsequent experimental periods. There is the possibility, however, that the preliminary period of protein feeding was not of sufficient length before the collection periods were started in these experiments. In no case, however, was there a preliminary period of less than 10 days in length.

The biological values obtained in these experiments for linseed-meal proteins compare favorably with those reported by Mitchell and Hamilton (15). Working with swine, these investigators obtained values averaging 61 when the proteins were fed at a 9-percent level. Braman (2), working with rats, obtained much higher values, averaging 78 at an 8-percent protein level. Bethke and his associates (1) reported average values of 71 for linseed-meal proteins fed to rats at a 10-percent level. There is possibly a slight species difference in the utilization of proteins which may account for some of the differences obtained, but they can be attributed in part to the difference in level of protein intake.

It should also be pointed out that some dried yeast was provided in the rations used in these investigations (1, 2, 15) which undoubtedly furnishes protein of high quality. This protein might supplement that from the feeds in question.

For soybean-oil-meal proteins fed with corn silage and corn to lactating cows, Holdaway, Ellett, and Harris (8) reported utilization values of 77 percent. Mitchell and Villegas (18) reported average biological values of 64 for the proteins in soybeans fed to rats at a 10-percent level. These values, however, are not directly comparable to those obtained in the experiments reported in this paper. Many practical feeding experiments have been conducted, however, which show the high value of soybean-oil meal as a protein-rich supplement.

TABLE 4.—Nitrogen metabolism data showing the digestibility and biological value of proteins in the first experiment

LOW-NITROGEN RATION																	
Wether no.	Body weight			Food intake	Dry-matter intake	Nitro-gen intake	Fecal nitro-gen	Esti-mated meta-bolic nitro-gen	Food nitro-gen in feces	Ab-sorbed nitro-gen	Nitro-gen in urine	Endoge-nous nitro-gen in urine	Food nitro-gen uti-lized	Dires-tion coef-ficient	Total nitro-gen stored	Digesti-ble nitro-gen stored	Biologi-cal value
	Initial	Final	Aver-age														
Kilo-gram	Kilo-gram	Kilo-gram	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Percent	Percent	Percent	Percent
6	19.40	20.37	19.89	3,678	3,561.9	6.31	19.78	10.555			8.30	10.417					
7	19.60	17.30	18.45	3,573	3,458.6	6.45	23.78	1.688			8.60	3.466					
8	16.20	14.87	15.54	2,000	1,938.0	3.39	12.47	1.643			9.17	3.580					
CORN-GLUTEN-MEAL RATION																	
6	22.87	23.90	23.39	6,997	6,655.5	123.01	41.19	36.67	4.52	118.49	40.35	9.01	31.34	87.15	67	34	51
7	28.47	29.03	28.75	8,000	7,852.4	142.40	51.47	41.85	9.62	132.78	54.26	9.06	45.20	87.58	64	26	40
8	22.43	23.33	22.88	8,000	7,706.4	139.68	41.84	47.63	1.00	139.68	64.53	10.75	53.78	85.90	70	24	34
LINSEED-MEAL RATION																	
6	23.90	24.37	24.14	7,000	6,664.7	122.64	50.97	36.46	14.51	108.13	47.56	5.62	38.94	69.19	58	20	34
7	23.10	23.77	23.44	8,000	7,603.2	141.12	54.54	48.13	6.41	134.71	46.24	9.49	36.75	97.96	61	29	47
8	24.27	25.43	24.85	8,000	7,595.0	142.16	46.01	46.03	1.00	142.16	53.64	10.19	43.65	98.51	68	30	44
SOYBEAN-OIL-MEAL RATION																	
6	26.33	26.80	26.57	7,000	6,606.6	122.22	40.38	35.94	4.44	117.78	40.11	8.74	31.37	86.41	67	34	51
7	26.10	27.00	26.55	8,000	7,660.8	140.40	49.06	45.35	3.71	136.69	43.74	9.56	34.18	102.51	65	34	52
8	19.87	20.77	20.32	8,000	7,622.4	141.68	40.95	48.10	1.00	141.68	45.17	10.77	34.40	107.28	71	39	55

LOW-NITROGEN RATION

6	25.83	23.57	24.70	3,511	3,313.2	7.69	17.88	10.540	7.39	10.269		
7	29.23	27.83	28.53	4,630	4,369.7	10.31	22.26	12.509	7.66	12.268		
8	25.30	24.00	24.65	4,000	3,778.8	8.74	22.42	12.593	8.54	13.346		

¹ Totals are for 10-day experimental periods.

² Fecal nitrogen per 100 g dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

³ Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in the case of metabolic nitrogen in the feces.

⁴ Estimated metabolic nitrogen greater than fecal nitrogen, therefore it was assumed that no food nitrogen was present in the feces.

TABLE 5.—Nitrogen metabolism data showing the digestibility and biological value of proteins in the second experiment¹

LOW-NITROGEN RATION																		
Wether no.	Body weight			Food intake Grams	Dry-matter intake Grams	Nitro- gen intake Grams	Fecal nitro- gen Grams	Esti- mated meta- bolic nitro- gen	Food nitro- gen in feces Grams	Ab- sorbed nitro- gen Grams	Nitro- gen in urine Grams	Endoge- nous nitro- gen in urine Grams	Food nitro- gen in urine Grams	Food nitro- gen uti- lized Grams	Diges- tion coef- ficient	Total nitro- gen stored Percent	Digesti- ble nitro- gen stored Percent	Biologi- cal value
	Initial	Final	Average															
1	25.83	23.57	24.70	3,511	3,313.2	7.69	17.88	2,054.0			7.30	2,029.0						
2	26.23	27.83	28.53	4,630	4,369.7	10.31	22.26	2,509			7.66	2,268						
3	25.30	24.00	24.65	4,000	3,778.8	8.74	22.42	2,593			8.54	2,546						
CORN-GLUTEN-MEAL RATION																		
1	33.80	34.90	34.35	8,000	7,426.4	138.88	44.66	37.43	7.23	131.65	61.38	10.72	50.66	80.99	68	24	35	62
2	31.93	33.47	32.70	8,184	7,774.3	144.70	50.44	39.73	10.71	133.99	48.72	7.78	40.94	93.05	65	31	48	69
3	29.00	30.77	29.89	9,000	8,467.0	158.76	57.38	58.22	4.00	158.76	69.91	9.59	60.32	98.44	64	20	31	62
LINSEED-MEAL RATION																		
1	31.03	32.10	31.57	8,000	7,432.8	142.64	47.12	38.13	8.99	133.65	48.44	9.76	38.68	94.97	67	33	49	71
2	37.43	37.70	37.57	8,200	7,631.4	146.63	49.02	39.25	9.77	130.86	62.78	7.78	55.00	75.86	65	21	31	71
3	27.67	28.83	28.35	9,000	8,367.1	158.76	62.39	56.03	6.36	152.40	54.13	9.33	44.80	107.60	61	27	44	71
SOYBEAN-OIL-MEAL RATION																		
1	28.33	30.27	29.30	8,000	7,594.0	141.20	51.38	39.65	11.73	129.47	35.59	8.97	26.62	102.85	64	38	60	79
2	34.87	35.93	35.40	8,200	7,662.9	143.91	45.79	39.25	6.56	137.35	57.27	7.89	40.38	87.97	68	28	42	79
3	31.43	32.67	32.05	9,000	8,458.2	155.43	51.92	60.65	4.00	155.43	56.28	10.00	46.28	109.15	67	30	46	70

LOW-NITROGEN RATION

6	34.53	31.97	33.25	4,000	3,827.6	6.61	18.92	± 0.494	10.52	± 0.316			
7	38.70	37.63	38.17	4,750	4,545.3	7.85	23.35	± 514	7.31	± 1.192			
8	28.17	29.13	28.65	3,550	3,684.1	6.36	27.82	± 755	8.65	± 1.302			

¹ Totals are for 10-day experimental periods.

² Fecal nitrogen per 100 g dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

³ Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in the case of metabolic nitrogen in the feces.

⁴ Estimated metabolic nitrogen greater than fecal nitrogen, therefore it was assumed that no food nitrogen was present in the feces.

TABLE 6.—*The digestibility and biological value of the proteins of corn-gluten meal, linseed meal, and soybean-oil meal when fed to lambs*

Item	Percentage digestibility and biological value when fed to lamb no. —						Average
	6 in experi- ment—		7 in experi- ment—		8 in experi- ment—		
	1	2	1	2	1	2	
Corn-gluten meal:							
Apparent digestibility.....	67	68	64	65	70	64	66.3±0.67
Percentage nitrogen intake stored.....	34	24	26	31	24	20	26.5±1.41
Percentage digested nitrogen stored.....	51	35	40	48	34	31	39.8±2.23
Biological value.....	74	62	66	69	61	62	65.7±1.40
Linseed meal:							
Apparent digestibility.....	58	67	61	65	68	61	63.3±1.08
Percentage nitrogen intake stored.....	20	33	29	21	30	27	26.7±1.42
Percentage digested nitrogen stored.....	34	49	47	31	44	44	41.5±2.01
Biological value.....	64	71	73	58	69	71	67.7±1.55
Soybean-oil meal:							
Apparent digestibility.....	67	64	65	68	71	67	67.0±.67
Percentage nitrogen intake stored.....	34	38	34	28	39	30	33.8±1.19
Percentage digested nitrogen stored.....	51	60	52	42	55	46	51.0±1.76
Biological value.....	73	79	75	64	76	70	72.8±1.45

The values for corn-gluten meal may, in a general way, be compared with those reported for corn grain since the gluten is largely zein, which comprises about 60 percent of the protein of corn grain. Mitchell and Kick (16) reported an average biological value of 54 for corn protein when fed to pigs at a protein level of approximately 8 to 9 percent. Later experiments with pigs by Mitchell and Hamilton (15) gave corn proteins an average biological value of 61 when fed at a protein level of 8.66 percent.

It should be pointed out that there is a possibility that the small amounts of wheat-straw nitrogen may have supplemented any amino acid deficiencies which any of the three feeds used in this investigation may have. As determined, however, the average values for soybean-oil meal are fairly high. There are no indications of marked amino acid deficiencies in this feed for sheep. These values are of special interest, since sheep have a high requirement for cystine and soybeans have been reported as deficient in this amino acid. However, Csonka and Jones (3, 4) have presented analytical data which do not support the idea of a quantitative deficiency of cystine in soybeans. They do admit however, the possibility of a deficiency in quality of protein, due to low availability of amino acids when the raw meal is fed. Osborne and Mendel (20) had earlier found the nitrogen of commercial soybean cake and of soybean meal cooked in water was utilized somewhat better than was the case with the raw and dry-heated meals. These writers believed the utilization of protein was increased by making the amino acids more available for assimilation. Perhaps expeller-process soybean meal would not show the cystine deficiencies which raw soybeans have apparently shown in nutrition experiments (17, 21).

Some observations on the fleeces of the lambs are of interest. The lambs were shorn at the beginning and again at the end of the experimental work. An examination of the wool fibers of all three fleeces showed "breaks" and distinct weakened places which corresponded exactly with the periods of low-nitrogen feeding. The

breaking strength and diameter of the wool fibers was greatly reduced during the periods of low-nitrogen feeding. The portions of the wool fibers grown during the periods of protein feeding were strong. The wool had a beautiful luster and the fibers were apparently of normal length.

SUMMARY

Metabolism studies were conducted with three growing wether lambs to determine the digestibility, storage, and biological value of the proteins of soybean-oil, corn-gluten, and linseed meals. The experiment was repeated, thus giving six determinations for each feed. Each of the feeds in question was added to a low-nitrogen ration in such amounts as to furnish a protein level of 10 percent, with approximately 1 percent additional being furnished by the other ingredients of the ration. All rations were equalized in energy content.

The average coefficients of apparent digestibility for protein were 67.0 percent for soybean-oil meal, 66.3 for corn-gluten meal, and 63.3 percent for linseed meal.

The lambs were more efficient in storing protein from the soybean-oil-meal ration than from either of the other rations. The average percentage of protein intake stored was 33.8 for soybean-oil-meal, 26.5 for corn-gluten meal, and 26.7 for linseed meal.

Slightly but significantly higher biological values were obtained for the soybean-oil-meal ration. The average of the biological values was 72.8 for soybean-oil-meal proteins, 65.7 for corn-gluten-meal proteins, and 67.7 for linseed meal proteins.

These data show the superiority of the proteins of soybean-oil meal over those furnished by linseed meal and corn-gluten meal. Furthermore, they indicate that it is possible to measure differences in quality of protein using sheep and the nitrogen-balance type of experimentation.

LITERATURE CITED

- (1) BETHKE, R. M., BOHSTEDT, G., SASSAMAN, H. L., KENNARD, D. C., and EDINGTON, B. H.
1928. THE COMPARATIVE NUTRITIVE VALUE OF THE PROTEINS OF LINSEED MEAL AND COTTONSEED MEAL FOR DIFFERENT ANIMALS. *Jour. Agr. Research* 36: 855-871, illus.
- (2) BRAMAN, W. W.
1931. THE RELATIVE VALUES OF THE PROTEINS OF LINSEED MEAL AND COTTONSEED MEAL IN THE NUTRITION OF GROWING RATS. *Jour. Nutrition* 4: 249-259.
- (3) CSONKA, F. A., and JONES, D. B.
1933. DIFFERENCES IN THE AMINO ACID CONTENT OF THE CHIEF PROTEIN (GLYCININ) FROM SEEDS OF SEVERAL VARIETIES OF SOYBEAN. *Jour. Agr. Research* 46: 51-55.
- (4) ——— and JONES, D. B.
1934. THE CYSTINE, TRYPTOPHANE, AND TYROSINE CONTENT OF THE SOYBEAN. *Jour. Agr. Research* 49: 279-282.
- (5) HART, E. B., and HUMPHREY, G. C.
1915. THE RELATION OF THE QUALITY OF PROTEINS TO MILK PRODUCTION. *Jour. Biol. Chem.* 21: 239-253, illus.
- (6) ——— and HUMPHREY, G. C., with the cooperation of SCHAAL, A. A.
1916. FURTHER STUDIES ON THE RELATION OF THE QUALITY OF PROTEINS TO MILK PRODUCTION. *Jour. Biol. Chem.* 26: 457-471, illus.
- (7) ——— and HUMPHREY, G. C., with the cooperation of SURE, B.
1917. THE RELATION OF THE QUALITY OF PROTEINS TO MILK PRODUCTION. *Jour. Biol. Chem.* 31: 445-460, illus.

- (8) HOLDAWAY, C. W., ELLETT, W. B., and HARRIS, W. G.
1925. THE COMPARATIVE VALUE OF PEANUT MEAL, COTTONSEED MEAL, AND SOYBEAN MEAL AS SOURCES OF PROTEIN FOR MILK PRODUCTION. *Va. Agr. Expt. Sta. Tech. Bull.* 28, 54 pp., illus.
- (9) LOVE, H. H.
1924. A MODIFICATION OF STUDENT'S TABLE FOR USE IN INTERPRETING EXPERIMENTAL RESULTS. *Jour. Amer. Soc. Agron.* 16: 68-73.
- (10) MCCOLLUM, E. V.
1914. THE VALUES OF THE PROTEINS OF THE CEREAL GRAINS AND OF MILK FOR GROWTH IN THE PIG, AND THE INFLUENCE OF THE PLANE OF PROTEIN INTAKE ON GROWTH. *Jour. Biol. Chem.* 19: 323-333.
- (11) MAYNARD, L. A., MILLER, R. C., and KRAUSS, W. E.
1928. STUDIES OF PROTEIN METABOLISM, MINERAL METABOLISM, AND DIGESTIBILITY, WITH CLOVER AND TIMOTHY RATIONS. N. Y. (Cornell) *Agr. Expt. Sta. Mem.* 113, 33 pp.
- (12) MITCHELL, H. H.
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. *Jour. Biol. Chem.* 58: 873-903.
- (13) ———
1924. THE BIOLOGICAL VALUE OF PROTEINS AT DIFFERENT LEVELS OF INTAKE. *Jour. Biol. Chem.* 58: 905-922.
- (14) ———
1928. A NOTE ON QUANTITATIVE METHODS OF MEASURING THE NUTRITIVE VALUE OF PROTEINS. *Biochem. Jour.* 22: [1323]-1326.
- (15) ——— and HAMILTON, T. S.
1931. THE NUTRITIVE VALUE FOR GROWING SWINE OF THE PROTEINS OF LINSEED MEAL AND OF COTTONSEED MEAL, BOTH ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN. *Jour. Agr. Research* 43: 743-748.
- (16) ——— and KICK, C. H.
1927. THE SUPPLEMENTARY RELATION BETWEEN THE PROTEINS OF CORN AND OF TANKAGE DETERMINED BY METABOLISM EXPERIMENTS ON SWINE. *Jour. Agr. Research* 35: 857-864.
- (17) ——— and SMUTS, D. B.
1932. THE AMINO ACID DEFICIENCIES OF BEEF, WHEAT, CORN, OATS, AND SOYBEANS FOR GROWTH IN THE WHITE RAT. *Jour. Biol. Chem.* 95: 263-281, illus.
- (18) ——— and VILLEGAS, V.
1923. THE NUTRITIVE VALUE OF THE PROTEINS OF COCONUT MEAL, SOYBEANS, RICE BRAN, AND CORN. *Jour. Dairy Sci.* 6: 222-236.
- (19) MORRIS, S., and WRIGHT, N. C.
1933. THE NUTRITIVE VALUE OF THE PROTEINS FOR MILK PRODUCTION. II. A COMPARISON OF THE PROTEINS OF BLOOD MEAL, PEA MEAL, DECORTICATED EARTH-NUT CAKE, AND A MIXTURE OF DECORTICATED EARTH-NUT CAKE AND FLAKED MAIZE. *Jour. Dairy Research* 5: 1-14, illus.
- (20) OSBORNE, T. B., and MENDEL, L. B., with the cooperation of FERRY, E. L., and WAKEMAN, A. J.
1917. THE USE OF SOYBEAN AS FOOD. *Jour. Biol. Chem.* 32: 369-387, illus.
- (21) SHREWSBURY, C. L., and BRATZLER, J. W.
1933. CYSTINE DEFICIENCY OF SOYBEAN PROTEIN AT VARIOUS LEVELS, IN A PURIFIED RATION AND AS A SUPPLEMENT TO CORN. *Jour. Agr. Research* 47: 889-895.
- (22) TURK, K. L., MORRISON, F. B., and MAYNARD, L. A.
1934. THE NUTRITIVE VALUE OF THE PROTEINS OF ALFALFA HAY AND CLOVER HAY WHEN FED ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN. *Jour. Agr. Research* 48: 555-570.
- (23) WOODWARD, J. C., and McCAY, C. M.
1932. SYNTHETIC DIETS FOR HERBIVORA. *Soc. Expt. Biol. and Med. Proc.* 30: 241-242.

NUTRIENT-SOLUTION PURIFICATION FOR REMOVAL OF HEAVY METALS IN DEFICIENCY INVESTIGATIONS WITH *ASPERGILLUS NIGER*¹

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INTRODUCTION

The difficulties associated with the demonstration in culture of the chemical elements required by organisms, especially as concerns those necessary in very small quantity, are great enough to make welcome the introduction of any simple procedure whereby an increase in sensitivity or precision may be accomplished. Commercial chemicals, even those of the highest purity, usually contain variable but appreciable traces of impurities, often sufficient in quantity to completely mask the requirement by the organism of the elements present as contaminants. Frequent attempts to improve the purity of commercial chemicals by recrystallization in the laboratory have met with but partial success or with failure. The contradictory and indecisive results often obtained with commercial chemicals and with compounds recrystallized in the laboratory have not aided in establishing definitely the heavy-metal requirements of organisms.

The successful application by the writer (8, 9)² of the method of nutrient-solution purification to the study of the heavy-metal requirements of the fungus *Aspergillus niger* Van Tiegh. has rendered desirable a thorough study of the method with a view to obtaining further improvement in results and a better understanding of the principles involved, so as to permit the direct application of the method to similar studies with green plants. The method of purification referred to consists in treating the nutrient solution with calcium carbonate in order to increase its alkalinity and to furnish calcium for the formation of precipitate to adsorb the traces of the heavy-metal precipitates simultaneously formed. In this way the heavy-metal impurities of the nutrient solution are precipitated and removed as a whole. It was found, for example, that the addition of an iron or zinc salt brought about a fivefold increase in yield of the fungus when compounds recrystallized in the laboratory were employed in the nutrient solution, whereas the increase obtained by such additions after the method of nutrient-solution purification with calcium carbonate had been employed was fiftyfold, or approximately 10 times as great.

Analytical methods for the determination of the heavy metals (iron, zinc, copper, and manganese) unfortunately are of insufficient sensitivity or precision to permit of determining the effectiveness of the method of purification. While in exceptional cases, as with iron, these methods permit of the detection of concentrations of about 0.1

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² Reference is made by number (*italic*) to Literature Cited p. 424.

mg of cation per liter, the apparent optima of these elements for growth of the fungus are approximately only 0.20, 0.14, 0.06, and 0.03 mg per liter, respectively. Distinct responses in growth that may be duplicated with fair precision are easily obtainable with variations in metal concentration of 0.01 mg of cation per liter. The addition to the nutrient solution of 0.001 mg of zinc per liter (1 part per billion, or $0.05 \gamma^3$ per culture of 50 cc) may bring about an increase of 92.5 percent in yield of *Aspergillus niger*.

The very minute quantities in which these elements are required do not denote their importance to the organism, since the absence of one of the elements from the nutrient solution may result in lowering the yield by 60 to 98 percent. The high dilutions in which these elements seem to be effective perhaps are likewise deceptive, inasmuch as their absorption by the cell may result in their concentration within the protoplast.

Modifications of the writer's method of nutrient-solution purification have been proposed. Bortels' (1) studies in effect demonstrate that if the nutrient solution is first made alkaline absorbent charcoal may be substituted for the calcium carbonate in the method of nutrient-solution purification. Bortels obtained results similar to those secured by the writer on the iron and zinc requirements of the fungus, but he included copper also as essential for the organism. Roberg (?), on the other hand, was able to show that alkalinity alone is sufficient to precipitate most of the traces of heavy-metal impurities in the nutrient solution. Furthermore, according to Roberg, iron, zinc, and copper are essential to *Aspergillus niger*. Another paper by the writer (11), besides confirming the foregoing results with iron, zinc, and copper, presents evidence that manganese also is essential to the mold.

A recent paper by Hopkins (5) describes a successful application of the method of nutrient-solution purification for the removal of manganese in studies with algae and with *Lemna*. Growth-manganese ratios of 11 or more were obtained by this author. Instead of using calcium carbonate to increase the alkalinity as well as to supply calcium for the formation of precipitate, Hopkins, like Bortels, employed an alkaline solution but added calcium nitrate instead of charcoal. Emerson (3), in his investigations on photosynthesis, found the calcium carbonate procedure helpful in regulating chlorophyll concentration by adjustment of the iron content of the nutrient solution.

Supplementing the present work on the calcium carbonate method of nutrient-solution purification for the removal of heavy metals, data are presented on some of its possible modifications. A limited investigation also was made respecting the possibility of extracting any heavy metals stored in the spores prior to their use for the inoculation of cultures.

METHODS

The methods employed in these studies are similar in all essentials to those previously employed and described. The same "W" strain of *Aspergillus niger* was grown for 6 days, at 34.7° to 35° C., on 50 cc of 5-percent sucrose solution to which the necessary salts had been added, in 200-cc pyrex Erlenmeyer flasks. The incubator employed

³ $1 \gamma = 1 \mu\text{g}$ (microgram).

has been described elsewhere (10). Inoculation was by means of a spore suspension containing 0.5 g of potassium bicarbonate per liter, the inoculum amounting to approximately 0.05 mg of spore material per flask. The cultures were filtered, when harvested, through fritted glass crucibles of porosity 3 and of 35-cc capacity. Instead of drying the felts for 4 days at 103° to 105°, they were dried for 2 hours in a rapid current of dry air at 50° to 70° and then overnight in an oven at 103° to 105°. Compressed air at 60 to 70 pounds' pressure was used instead of a blower and proved satisfactory because of its low relative humidity on expansion. This procedure was found very satisfactory not only in saving time but also in preventing fouling of the crucibles and in preserving the felts in their natural colors.

The nutrient solutions, after treatment and filtration through fritted glass crucibles of porosity 4, were distributed into the Erlenmeyer flasks, and the heavy metals, in solution as sulphate, were added with a pipette to the individual flasks. The flasks then were sterilized by steaming for 10 minutes.

Acidity readings were obtained with a potentiometer and quinhydrone electrode sensitive to 0.01 pH.

NUTRIENT-SOLUTION PURIFICATION WITH CALCIUM CARBONATE

The method of nutrient-solution purification, as employed by the writer (8) for the removal of the last traces of iron and zinc from the complete nutrient solution, has several advantages as compared with purification by recrystallization. Instead of a series of operations with each individual compound, the method of nutrient-solution purification requires only two operations and deals with the impurities of the nutrient compounds as a whole. As might be anticipated, it is not only much simpler and more rapid than recrystallization but is also more effective. The decrease in time and in the number of utensils employed is also an important factor in securing absence of contamination and uniformity of results.

The original procedure employed in nutrient-solution purification was to autoclave the complete solution with 15 g of calcium carbonate per liter for 20 minutes at a pressure of 1 atmosphere (120.5° C.). After standing overnight the solution was decanted from the sediment containing the iron and zinc and distributed into culture flasks.

The results of a study of this method for the purpose of determining the effect of the different factors entering into its operation have been tabulated in table 1. The growth ratio, which is determined by dividing the yield obtained in the presence of an element by the yield obtained when this element is omitted, is the reciprocal of the percentage of maximum yield. Duration of treatment has reference to the time elapsing between heat treatment and filtration. The table discloses that variation in the concentration of the nutrient solution or purificant, in the duration of treatment, in the acidity, and in the use of ammonium sulphide did not lead to any marked improvement in results. This is also true in regard to the length of time for which the solution was heated with calcium carbonate and for the separate treatment of sucrose and salts. However, decreasing to a minimum the total amount of nutrient salts per liter led to a slight improvement in results. Filtration immediately following treatment seems advisable. The method, therefore, is not critical with respect to the factors entering into its operation.

TABLE 1.—Effect of variations in purification method with calcium carbonate on growth of *Aspergillus niger* in a nutrient solution containing 5 percent sucrose

Culture no.	Content in nutrient solution			CaCO ₃	Duration of treatment	Initial pH	Yield without heavy metals	Growth ratio with—					Maximum individual yield
	NH ₄ NO ₃	KH ₂ PO ₄	MgSO ₄ ·7H ₂ O					Fe	Zn	Cu	Mn	All	
	Grams per liter	Grams per liter	Grams per liter	Percent	Hours		Mg						Mg
1	10.0	5.0	2.5	1.5	24	6.64	22.2	12.46	25.09	1.13	1.30	48.93	1,110.9
2	2.1	.75	.55			4.73	208.9	2.15	2.98	1.02	1.66	4.41	938.7
3	2.5	1.0	1.0	3.0	27	7.12	18.6	49.25	16.12	1.76	1.25	55.59	1,056.1
4	2.5	1.0	1.0	1.5	24	6.90	19.4	32.17	17.20	1.48	1.14	46.93	1,000.8
5	2.5	1.0	1.0	1.5	24	6.77	14.2	41.34	34.98	1.67	1.08	67.24	1,040.6
6 ¹	2.5	1.0	1.0	1.5	24	7.11	17.5	28.79	27.66	1.39	1.09	48.21	913.8
7 ²	2.5	1.0	1.0	.5	24	6.73	23.1	85.11	23.16	1.54	1.13	40.90	1,007.0
8	2.5	1.0	1.0	.5	24	6.87	10.3	33.50	37.17	1.60	1.20	75.79	822.5
9	2.5	1.0	1.0	.1	24	6.47	17.4	32.03	32.26	1.37	1.24	52.28	1,009.7
10 ³	2.5	1.0	1.0	.1	0	6.29	15.8	62.83	39.21	1.21	2.13	64.03	1,011.6
11 ⁴	2.5	1.0	1.0	.1	0	5.72	23.2	38.47	36.25	1.14	1.76	45.94	1,078.9
12	2.5	1.0	1.0	.1	48	6.65	16.1	43.35	12.19	1.58	1.49	63.27	1,030.2
13	2.5	1.0	1.0	.1	75	6.63	28.3	30.28	8.98	1.13	1.33	35.52	1,030.7
14 ⁵	2.5	1.0	1.0	.1	24	6.34	24.1	44.98	17.60	1.17	1.30	43.30	1,043.5
15	5.0	1.0	1.0	.1	26	6.46	19.9	41.90	12.62	1.51	1.37	52.43	1,046.4
16	2.5	2.0	1.0	.1	26	6.32	15.5	43.63	38.15	1.79	1.31	61.08	964.2
17	2.5	1.0	2.0	.1	26	6.49	18.3	80.00	38.82	1.13	1.08	57.27	1,095.3
18 ⁶	2.5	1.0	1.0	.1	28	6.90	19.4	45.97	35.71	1.33	1.43	49.52	997.5
19	2.5	(⁷)	1.0	.1	28	6.95	11.8	64.87	50.56	1.10	1.37	87.41	1,039.0
20	2.5	(⁸)	1.0	.1	28	7.81	12.4	60.57	42.53	1.07	1.60	64.48	813.1

¹ Treatment at × 20 concentration, then diluted.² Treatment at × 5 concentration, then diluted.³ Filtered immediately after treatment.⁴ Sucrose and salts treated separately.⁵ Autoclaved 1 hour.⁶ Added 0.5 cc (NH₄)₂S per liter.⁷ Substituted 0.64 g of K₂HPO₄.⁸ Substituted 0.52 g of K₂PO₄.

Attempts to obtain an improvement by the use of filter pulp and excess iron (5 mg Fe per liter) were futile and gave the same results as those tabulated. The additional adsorptive capacity of the filter pulp and of the precipitate of iron salt seemed unnecessary. Another experiment with a solution which on mixing had a pH value of 7.19, and was filtered to remove the very slight precipitate formed, gave growth ratios of 16.91, 4.64, 1.09, and 1.20 for iron, zinc, copper, and manganese, respectively. These ratios, while equal to or superior to those obtained with the untreated solution, are inferior to those obtained with the treated solution, an indication that the adsorptive capacities of the precipitates formed during purification play a necessary part in the process.

Also of interest are the results obtained on the sporulation of the fungus and the accompanying variations in acidity of the nutrient solution resulting from the growth of the organism. This information has been tabulated in table 2. It will be noted that sporulation is inhibited if any of the four heavy metals under discussion are omitted from the nutrient solution. In the absence of copper, moreover, the deposition of pigment in the spore walls is interfered with and the spores are brown, yellow, or white, apparently according to the extent of the deficiency of this metal in the nutrient solution.

TABLE 2.—*Sporulation of Aspergillus niger and pH of nutrient solution at harvest with calcium carbonate employed as purificant*

Culture no.	Sporulation ¹ in absence of indicated heavy metals						pH at harvest in absence of indicated heavy metals					
	All	Fe	Zn	Cu	Mn	None	All	Fe	Zn	Cu	Mn	None
1.	1, bl	2, bl	1, bl	2, br	3, bl	4, bl	3.75	3.44	3.46	1.57	1.56	1.56
2.	2, bl	3, bl	4, bl	4, br	0	5, bl	2.75	1.91	1.91	2.21	1.77	1.91
3.	1, bl	1, bl	2, bl	2, y	3, br	5, bl	3.01	3.02	2.90	2.24	1.63	2.64
4.	1, bl	2, bl	3, bl	4, br	4, bl	5, bl	3.20	2.98	2.87	1.89	1.85	2.10
5.	1, bl	2, bl	2, bl	3, br	4, bl	5, bl	3.22	3.02	2.85	1.94	1.91	2.11
6.	1, bl	3, bl	3, bl	4, br	5, bl	5, bl	2.97	2.77	2.77	1.78	1.79	1.93
7.	2, bl	2, bl	3, bl	3, br	3, bl	4, bl						
8.	2, bl	2, bl	3, bl	3, br	5, bl	5, bl	3.20	3.01	2.85	1.83	1.85	1.93
9.	2, bl	2, bl	3, bl	3, br	5, bl	5, bl	3.20	2.89	2.88	1.96	1.85	2.01
10.	1, y	1, y	1, y	2, y	0	5, y	2.95	2.89	2.98	1.78	1.53	1.72
11.	1, y	1, y	2, y	2, y	0	3, bl	3.01	2.91	2.93	1.79	1.52	1.65
12.	1, bl	1, bl	2, bl	2, y	2, bl	4, bl	3.19	2.99	2.89	2.08	1.58	1.91
13.	1, bl	2, bl	5, bl	3, y	0	5, bl	3.35	3.02	2.65	2.64	1.50	2.44
14.	1, y	1, y	2, y	2, w	0	4, bl	3.07	2.73	2.76	2.10	1.50	2.01
15.	1, bl	1, bl	3, bl	3, br	2, bl	3, bl	3.29	3.07	2.80	1.50	1.46	1.37
16.	1, bl	1, bl	2, bl	3, y	3, bl	4, bl	3.21	3.01	3.06	1.94	1.64	
17.	1, bl	2, bl	2, bl	3, br	3, bl	4, bl	2.97	3.01	3.01	2.28	1.74	2.28
18.	1, bl	1, bl	1, bl	2, y	2, bl	4, br	3.15	3.05	3.08	1.94	1.60	1.84
19.	1, bl	1, bl	1, bl	2, br	2, br	4, bl	3.14	2.98	3.05	1.97	1.67	1.67
20.	1, bl	1, bl	1, bl	2, br	1, bl	5, bl	3.04	2.91	2.91	2.04	1.67	1.57

¹Amount of sporulation is indicated as 0 (sterile) to 5 (entirely covered with spores) and spore color by the initial letter or first 2 letters of the words jet, black, brown, tan, yellow, and white.

The presence in the nutrient solution of both iron and zinc seems to be necessary for the acid metabolism of the organism, whereas the addition of manganese and perhaps copper seems unnecessary. Manganese particularly does not seem to be required for acid formation and it is surmised that it bears some relation to the processes whereby the organic acids are decomposed. Since both copper and manganese are still present in the cultures in amounts sufficient for 50 to 75 percent of maximum growth, definite conclusions as yet cannot be drawn.

NUTRIENT-SOLUTION PURIFICATION WITH BASIC MAGNESIUM CARBONATE

The data of table 3 show the results obtained by the substitution of basic magnesium carbonate for calcium carbonate in freeing the nutrient solution from heavy metals. The results are similar to those obtained with calcium carbonate. However, the use of basic magnesium carbonate possesses certain advantages over that of calcium carbonate. It avoids the introduction of a nonnutrient, and heat treatment may be continued for 20 minutes in the steamer at 100° C. instead of in the autoclave at 120.5°, since the basic magnesium carbonate reacts quickly. Furthermore, the nutrient solution so purified seems to be nearly free from calcium and shows no trace of this element when tested with potassium fluoride or ammonium oxalate. Moreover, the absence of calcium to the degree indicated in these tests does not cause a decrease in the maximum yield. The disadvantage of the method is that it is critical for concentration of purificant employed, an excess resulting in the more or less complete removal of phosphate.

TABLE 3.—Effect of variations in purification method with basic magnesium carbonate upon growth of *Aspergillus niger* in a nutrient solution containing 5 percent sucrose¹

Culture no.	Content in nutrient solution			4MgCO ₃ ·Mg(OH) ₂ ·5H ₂ O Percent	Initial pH	Yield without heavy metals	Growth ratio with—					Maximum individual yield
	NH ₄ NO ₃	KH ₂ PO ₄	MgSO ₄ ·7H ₂ O				Fe	Zn	Cu	Mn	All	
	Grams per liter	Grams per liter	Grams per liter									
1.....	2.5	1.0	0.3	0.04	7.15	13.6	51.91	5.26	1.42	1.44	68.00	925.5
2.....	2.5	1.0	.3	.06	7.39	10.5	54.17	6.10	1.58	1.56	99.57	1,057.7
3.....	2.5	1.0	.3	.07	7.52	22.7	51.43	28.06	1.53	1.89	41.53	984.2
4.....	2.5	1.0	.3	.08	7.62	23.2	53.10	5.86	1.56	1.82	30.39	905.8
5 ²	2.5	1.0	.3	.07	8.15	11.1	11.55	20.20	.82	1.03	31.12	525.2
6.....	2.5	1.0	1.0	.10	7.60	7.7	52.46	7.32	1.61	1.14	16.35	190.4
7.....	5.0	1.0	1.0	.10	7.29	12.6	45.26	15.19	1.96	1.48	36.28	479.3
8.....	2.5	2.0	1.0	.10	6.94	12.5	33.26	4.97	1.49	1.46	76.37	991.6

¹ Duration of treatment was 24 hours in all cases

² Added 0.5 cc (NH₄)₂S per liter

The data on sporulation and acidity at harvest, obtained in the experiments tabulated in table 3, have been summarized in table 4. The results are essentially the same as those given with the calcium carbonate method. The effects of omission of heavy metals on sporulation seem to be intensified, however, while the variations in acidity remain about the same.

TABLE 4.—Sporulation of *Aspergillus niger* and pH of nutrient solution at harvest with basic magnesium carbonate employed as purificant

Culture no.	Sporulation in absence of indicated heavy metals ¹						pH at harvest in absence of indicated heavy metals					
	All	Fe	Zn	Cu	Mn	None	All	Fe	Zn	Cu	Mn	None
1.....	1, bl	2, bl	4, bl	3, y	1, bl	4, bl	2.96	2.93	2.37	1.95	1.71	1.73
2.....	1, bl	2, bl	4, bl	3, y	1, bl	4, bl	3.27	3.03	2.42	2.22	1.68	2.22
3.....	1, bl	1, bl	2, bl	2, y	2, bl	4, bl	3.28	3.00	3.04	1.83	1.63	1.78
4.....	1, bl	1, bl	4, bl	2, br	1, bl	3, bl	3.14	3.05	2.44	1.81	1.64	1.81
5.....	1, bl	1, bl	1, bl	1, bl	0	2, bl	2.29	3.08	3.16	1.85	1.74	1.81
6.....	0	0	2, bl	2, y	2, bl	3, bl	6.88	3.35	2.50	2.23	2.23	2.26
7.....	0	1, bl	2, bl	1, br	1, bl	3, bl	3.84	3.24	2.93	1.99	2.25	1.95
8.....	0	2, bl	5, bl	2, br	1, bl	5, bl	3.08	3.05	2.49	2.02	1.60	2.02

¹ See footnote 1, table 2.

NUTRIENT-SOLUTION PURIFICATION WITH ADSORBENT CHARCOAL

The experimental work with charcoal as purificant given in table 5 was planned primarily to ascertain whether the use of adsorbent charcoal in conjunction with a purificant of definite chemical composition would lead to any improvement. Basic magnesium carbonate was selected instead of calcium carbonate because the lower initial acidities brought about through its use are nearer the acidity

(pH 8) recommended for utilization of charcoal. Other compounds of the alkaline earth metals, such as the oxides, hydroxides, or tri-basic phosphates, probably would operate similarly. One experiment with calcium carbonate also has been included for comparison. The charcoals compared are (A) Carbox E (carboraffin), (B) cane-sugar charcoal, (C) decolorizing charcoal, and (D) medicinal charcoal purified by acid. None of these charcoals was free from ash constituents; even the cane-sugar charcoal contained 2.07 percent of ash.

The results obtained by supplementing the action of basic magnesium carbonate with adsorbent charcoal do not differ in any important degree from those previously discussed. All indications point to the conclusion that the use of charcoal is advisable only if it is necessary to avoid the use of an alkaline earth carbonate, hydroxide, or oxide. The lack of uniformity in results with different charcoals is most apparent in the acidities at harvest. These variations of course are not unexpected, inasmuch as organic compounds and 2 to 5 percent of ash constituents are usually present in an adsorbent charcoal. Miller (6) asserts that ash-free charcoal is not an effective adsorbent for cations no matter what its source, and claims that adsorption of cations is largely a property of the contaminants present in the carbon.

EFFECT OF SPORE EXTRACTION UPON SUBSEQUENT GROWTH OF *ASPERGILLUS NIGER*

The inability to obtain growth ratios higher than 2 (50 percent of maximum yield) with copper and manganese in these investigations must be due to a technic inadequate to remove these metals entirely from the nutrient solution, to their introduction as contaminants with the heavy metals intentionally added, to the presence of these materials in the spores, or to a combination of these causes. The only alternative is the assumption that an element may be essential for a physiological function and yet not be of the first importance to growth. This alternative would seem improbable, however, inasmuch as other data on hand show that the absence of any of the elements essential to *Aspergillus niger* results in the almost complete suppression of growth. Hence it seemed advisable to attempt to determine experimentally the extent to which the growth of the fungus is influenced by the presence of copper and manganese in the spores. Parallel studies also were made on the effect of iron and zinc on growth.

Extraction of the spores with dilute alkali was adopted initially, since the black pigment (aspergillin) in the spores is known to contain iron and to be soluble in dilute alkalies. The possibility that the storage of copper and manganese in the spores is associated with that of iron rendered this procedure attractive for a first trial. Tests demonstrated that the pigment could be extracted from the spores with potassium bicarbonate (0.5 to 30.0 g per liter) without injury to the spores. Experiments in which ammonium hydroxide was employed also were performed. The results are tabulated in table 6. The increased growth ratios with iron, copper, and manganese would indicate the presence of these materials in the spores and the feasibility of at least their partial removal. The negative results with zinc are to be attributed to its presence in the potassium bicarbonate and ammonium hydroxide employed, since the use of redistilled ammonia led to a higher growth ratio with zinc.

TABLE 5.—*Development of Aspergillus niger in nutrient solutions purified with calcium carbonate or basic magnesium carbonate and with various adsorbent charcoals*¹ employed as supplementary purificants

Heavy metals omitted	0.1 percent CaCO ₃ and 0.1 percent (A)						0.07 percent 4MgCO ₃ ·Mg(OH) ₂ ·5H ₂ O and indicated percentages of various charcoals					
	0.1 percent (A)			0.5 percent (A)			0.5 percent (B)			0.5 percent (C)		
	Growth ratio	pH at harvest	Sporulation	Growth ratio	pH at harvest	Sporulation	Growth ratio	pH at harvest	Sporulation	Growth ratio	pH at harvest	Sporulation
All.....	17.0	5.80	1, bl	8.3	7.37	1, bl	13.3	7.34	1, bl	15.5	7.59	1, j
Fe.....	64.36	3.35	1, bl	118.78	3.36	1, bl	82.70	3.32	1, bl	66.54	3.23	1, j
Zn.....	51.13	2.95	1, bl	39.28	2.99	1, bl	52.38	3.09	1, bl	47.31	3.15	1, j
Cu.....	20.96	2.88	3, bl	64.02	3.06	1, bl	28.04	2.90	2, j	30.88	3.11	2, j
Mn.....	1.04	2.88	3, br	1.21	2.16	2, br	1.10	3.29	3, t	1.08	3.06	3, t
None.....	1.02	2.44	4, bl	1.22	1.85	2, bl	.97	2.65	4, b	1.54	1.65	0
	1, 100.0	2.46	4, bl	1, 124.8	3.07	3, bl	1, 181.7	2.79	4, bl	1, 053.9	3.58	4, bl

¹ Letters indicate the different charcoals as follows: A, Carbox E (carboraffin); B, cane-sugar charcoal; C, decolorizing charcoal; D, medical charcoal purified by acid.

² Average yield in milligrams without heavy metals.

³ Initial pH.

⁴ Maximum individual yield in milligrams.

TABLE 6.—*Effects of spore extraction on the development of Aspergillus niger in a 2.5–1.0–1.0 nutrient solution purified with 0.1 percent of calcium carbonate*

Heavy metals omitted	Spores extracted with KHCO_3 of indicated concentration						Spores extracted with NH_4OH of indicated concentration					
	0.5 percent			3.0 percent			1.0 percent			5.0 percent		
	Growth ratio	pH at harvest	Sporulation	Growth ratio	pH at harvest	Sporulation	Growth ratio	pH at harvest	Sporulation	Growth ratio	pH at harvest	Sporulation
All	1 23.0	2 6.77	1 bl	1 15.1	2 6.67	1 bl	1 18.8	2 5.98	1 1	1 14.5	2 6.65	1 12.7
Fe	41.06	2 9.99	2 bl	64.97	3.08	1 bl	49.13	3.15	1 1	45.12	3.21	65.32
Zn	39.84	2 9.99	2 bl	112.77	3.05	1 bl	46.18	2.85	1 1	30.71	3.06	45.83
Cu	20.66	2 7.9	2 bl	5.26	2.41	4 bl	7.87	2.39	2 1	28.44	3.12	33.18
Mn	1.99	1.81	3 bl	1.67	2.10	2 y	1.76	1.80	1 w	1.09	1.74	1.19
None	1.09	1.88	4 bl	1.38	1.74	2 bl	2.24	1.60	1 1	1.97	1.63	1.58
	1,040.3	2.01	5 bl	982.5	1.84	4 bl	923.6	1.92	3 1	654.2	1.65	826.5

1 Average yield in milligrams without heavy metals.

2 Initial pH.

3 Maximum individual yield in milligrams.

4 Purified by distillation.

DISCUSSION

To judge from the description by Britton (2) of the factory methods for the purification of cane sugar and the acidity at which the heavy metals are precipitated as phosphates, it would seem that the method of nutrient purification with calcium carbonate is quite similar to the factory procedure for the purification of sucrose. The heavy-metal impurities would appear to be precipitated largely as phosphates, though some may also separate out as hydroxide, carbonate, or basic carbonate, depending on the acidity of precipitation. On the assumption that a precipitate of the most insoluble compound is always formed, it is probable that under these conditions practically all the heavy metals will undergo precipitation in one form or another. Thus, when basic magnesium carbonate is used as the purificant even barium, strontium, and calcium probably will be removed if the proper quantity of purificant (and therefore the appropriate acidity) is utilized, since the salts of the latter elements are precipitated at a higher acidity than is magnesium. In principle the method seems dependent upon increasing the alkalinity of the solution in the presence of an alkaline earth sufficiently to cause the precipitation of the alkaline earth simultaneously with that of the heavy metal it is desired to remove. The precipitate of the alkaline earth compound thus serves as a "gatherer" or adsorbent. The chemistry of the process therefore is elementary. As suggested by Hopkins (5), perhaps also other cations, such as some of the heavy metals, may be found to answer the same purpose. It is immaterial whether the alkaline earth be added before or after the decrease in acidity, and the means whereby the decrease in acidity is brought about also is of no consequence. Neither is it important whether the purificant is added as an integral part of the nutrient solution.

The speed of reaction with calcium carbonate seems to be slower, and it is stated by Hillebrand and Lundell (4) that the limit of alkalinity corresponds to pH 7.4, whereas the basic magnesium carbonate reacts rapidly and can produce pH 9.5 if sufficient be added. With the former material the reaction never goes to completion under the conditions employed, while with the basic magnesium carbonate the reaction is always complete.

Since the precipitates formed during purification are never absolutely insoluble it cannot be claimed that the process completely removes the heavy metals. The efficiency of removal differs with the element and appears to be greater with iron and zinc than with copper or manganese. A puzzling feature in this connection is that whereas formerly the purified solution was always light yellow as the result of caramelization (8) the solutions in the present series of experiments were always colorless.

The results obtained through extraction of the spores of *Aspergillus niger* are distinctly encouraging and would seem to indicate the utility of this procedure as an experimental tool to accentuate heavy-metal deficiencies with the fungi. This procedure may prove feasible also with seed of green plants and appropriately selected compounds. The quantities of heavy metal stored in the spore or seed in some cases may be found sufficient in quantity to provide completely for the growth of the organism. This does not seem to be the case with *A. niger*, however.

Data obtained in an unsuccessful attempt to remove all halogens from the nutrient solution are illustrative of the magnitudes dealt with in these experiments and of the sensitivity of response of the organism. The addition of silver nitrate (5 mg Ag per liter) to the nutrient solution, followed by treatment with calcium carbonate to remove excess silver, gave upon filtration a solution presumably free of halogens. The presence of silver in the solution was revealed, however, through absence of growth upon inoculation. Nevertheless, a series of simultaneous cultures to which slight excesses of sodium chloride, sodium bromide, and sodium iodide had been added gave characteristic precipitates and yields of 0, 121.3, and 748.2 mg per culture, respectively. The solubilities of silver chloride, silver bromide, and silver iodide in water are 1.5, 0.11, and 0.003 mg per liter, respectively. On the assumption of equal solubility of silver iodide in water and in 5-percent sucrose, the results would indicate that 1.5 parts per billion of silver ion are sufficient to cause a loss of about 25 percent in yield. Actual determinations later gave decreases in yield of 9.9 and 6.4 percent with 1 part per billion of silver ion, though in unpurified solutions.

Without attempting to minimize the value of the method of nutrient-solution purification for biological study, nevertheless it must be admitted to be a makeshift and of value mainly because of the presence of excessive quantities of impurities in the commercial chemicals available for work of this character. Its disadvantage consists in the inability to predict the exact composition of a nutrient solution after treatment and consequently the limitations in its employment for critical study of nutrient proportions. It is estimated that a twentyfold improvement in the purity of commercial chemicals would permit one to dispense with the method of nutrient-solution purification in nutrition studies with plants. To be entirely satisfactory an increase of at least fiftyfold would be required in the purity of the compounds employed.

Pending the development of methods for producing chemicals that might be referred to as biologically pure and of adequate chemical or spectroscopic tests for ascertaining their purity, the calcium carbonate method of nutrient purification seems to be the best procedure available for the removal of the last traces of heavy metals. This method, or variants of it, has already been applied successfully to determine the heavy-metal requirements of various fungi, of algae, and of *Lemna*. Since the salts may be subjected to purification successfully in the absence of sucrose (table 1, culture no. 11) there is no reason to think that the method cannot be applied to the study of the heavy-metal requirements of the higher plants. Moreover, the agreement in results obtained with selected commercial chemicals and with the various modifications of the purification method here discussed makes it evident that these results on the requirements of *Aspergillus niger* for iron, zinc, copper, and manganese cannot be accidental and should serve definitely to establish these needs. These heavy metals have been claimed to be essential also to green plants and to animals. Results with the method of nutrient-solution purification obtained with quartz apparatus were essentially similar to those obtained with pyrex glassware.

SUMMARY

Purification of the nutrient solution for the removal of heavy metals may be accomplished by treating the nutrient solution with calcium carbonate and filtering while hot. The method is not critical and gives good results with iron, zinc, copper, and manganese under a wide variety of conditions and procedures. The removal of heavy metals appears to be dependent on their coprecipitation with an alkaline earth as phosphate, carbonate, or hydroxide by a decrease in acidity. The use of adsorbent charcoal to supplement the action of an alkaline-earth purificant is unnecessary and causes a decrease in experimental precision. Extraction of the spores of *Aspergillus niger* with alkaline solutions effects partial removal of iron, copper, and manganese stored in the spores in preparation for subsequent growth, and so leads to an accentuation of deficiency effects with these metals.

LITERATURE CITED

- (1) BORTELS, H.
1927. ÜBER DIE BEDEUTUNG VON EISEN, ZINK UND KUPFER FÜR MIKRO-ORGANISMEN. (UNTER BESONDERER BERÜCKSICHTIGUNG VON ASPERGILLUS NIGER.) Biochem. Ztschr. 182: [301]-358, illus.
- (2) BRITTON, H. T. S.
1932. HYDROGEN IONS, THEIR DETERMINATION AND IMPORTANCE IN PURE AND INDUSTRIAL CHEMISTRY. Ed. 2, rev. and enl., 589 pp., illus. London.
- (3) EMERSON, R.
1929. THE RELATION BETWEEN MAXIMUM RATE OF PHOTOSYNTHESIS AND CONCENTRATION OF CHLOROPHYLL. Jour. Gen. Physiol. 12: 609-622, illus.
- (4) HILLEBRAND, W. F., and LUNDELL, G. E. F.
1929. APPLIED INORGANIC ANALYSIS; WITH SPECIAL REFERENCE TO THE ANALYSIS OF METALS, MINERALS, AND ROCKS. 929 pp., illus. New York and London.
- (5) HOPKINS, F. F.
1934. MANGANESE AN ESSENTIAL ELEMENT FOR GREEN PLANTS. N. Y. (Cornell) Agr. Expt. Sta. Mem. 151, 40 pp., illus.
- (6) MILLER, E. J.
1927. ADSORPTION FROM SOLUTION BY ASH-FREE ADSORBENT CHARCOAL. III. A COMPARISON OF RESULTS OBTAINED WITH ASH-FREE AND IMPURE CHARCOAL. Jour. Phys. Chem. 31: 1197-1211.
- (7) ROBERG, M.
1928. ÜBER DIE WIRKUNG VON EISEN-, ZINK-, UND KUPFERSALZEN AUF ASPERGILLEN. Zentbl. Bakt. [etc.] (II) 74: 333-370, illus.
- (8) STEINBERG, R. A.
1919. A STUDY OF SOME FACTORS IN THE CHEMICAL STIMULATION OF THE GROWTH OF ASPERGILLUS NIGER. Amer. Jour. Bot. 6: 330-372.
- (9) ———
1934. THE SO-CALLED "CHEMICAL STIMULATION" OF ASPERGILLUS NIGER BY IRON, ZINC, AND OTHER HEAVY METAL POISONS. Bull. Torrey Bot. Club 61: 241-248.
- (10) ———
1934. A MODIFICATION IN INCUBATOR CONSTRUCTION. Phytopathology 24: 829-831, illus.
- (11) ———
1935. THE NUTRITIONAL REQUIREMENTS OF THE FUNGUS, ASPERGILLUS NIGER. Bull. Torrey Bot. Club 62: 81-90.

A SIMPLE, ACCURATE METHOD OF COMPUTING BASAL AREA OF FOREST STANDS¹

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INTRODUCTION

Basal area is a measure of a timber stand by the use of which the errors peculiar to measurement by volume are avoided, and by which a more accurate picture of the timber is provided than by diameter measurement alone. For these reasons it is widely used. The statistics of basal area which are commonly sought for various purposes are: The sum; the mean; the diameter of mean basal area; the standard deviation; the product moment, or sum of cross products; the growth of a tree; the growth of a stand or plot; the mean growth; and the standard deviation of growth. The common methods of obtaining these statistics are either exceedingly laborious or introduce systematic errors. The purpose of this paper is to present a method which is both precise and easy to apply.

REVIEW OF METHODS

The usual way of determining basal area of a timber stand is to list the basal area of every measured tree, using a basal-area table,² and compute the desired statistics from this list. Where there are many trees, this method is slow and laborious, but accurate in principle. In practice it lacks a systematic check, and errors creep in which may go unnoticed or may at best be troublesome to correct. With this method, the computation of any statistics besides the mean and sum of basal area and diameter of average basal area is not usually worth the effort.

Another common procedure in computing statistics of basal area is to group the measured diameters into classes and select a value for each class in using the basal-area table. This is obviously much less tedious than the other method and is also less liable to troublesome numerical mistakes. However, it introduces a systematic error due to grouping, the magnitude and direction of which depends on the diameter distribution and the class value chosen. Another class of error is introduced when the usual interpolation in the table is made by proportional parts; this error is always negative in sign.

THE FORMULA METHOD

The proposed method is derived from the analytical definition of basal area—

$$b = \frac{\pi d^2}{(4)(144)} \quad (1)$$

¹ Received for publication May 3, 1935; issued December, 1935.

² For simplicity, diameters are assumed to be perfect circles and without errors as measured.

where b is basal area in square feet and d is diameter in inches.

$$\text{If } k = \frac{\pi}{(4)(144)} = 0.005454 \dots$$

$$\text{this may be expressed: } b = kd^2 \quad (2)$$

Using this definition and the formulae which follow, computation of any of the above statistics of basal area is both easy and precise. This may be called the "formula method", to contrast with the usual table method.

APPLICATION TO UNGROUPED DATA

Computations of basal area by the formula method may be made with or without grouping. Ungrouped data generally mean a small sample, and with few trees and only simple statistics desired, the quickest procedure is to use the basal-area table. As the job increases in complexity the formula method becomes increasingly advantageous.

It readily follows from equation (2) that total basal area—

$$\Sigma b = k \Sigma d^2, \quad (3)$$

and that mean basal area—

$$\frac{\Sigma b}{N} = \frac{k}{N} \Sigma d^2 \quad (4)$$

where Σb is the sum and $\frac{\Sigma b}{N}$ mean of basal area, and Σd^2 is the sum of the squared diameters. The diameter of average basal area is

$$\begin{aligned} \text{from (2) and (4) above, } d' &= \sqrt{\frac{\Sigma b}{\frac{k}{N}}} \\ &= \sqrt{\frac{k \Sigma d^2}{\frac{k}{N}}} = \sqrt{\frac{\Sigma d^2}{N}} \end{aligned} \quad (5)$$

Standard deviation of basal area ³

$$\begin{aligned} \sigma_b &= \sqrt{\frac{\Sigma \left(b - \frac{\Sigma b}{N} \right)^2}{N}} = \sqrt{\frac{\Sigma b^2}{N} - \left(\frac{\Sigma b}{N} \right)^2} \\ &= \frac{1}{N} \sqrt{N \Sigma b^2 - (\Sigma b)^2} = \frac{k}{N} \sqrt{N \Sigma d^4 - (\Sigma d^2)^2} \end{aligned} \quad (6)$$

In small samples the standard deviation must be multiplied by

$$\sqrt{\frac{N}{N-1}}$$

³ An approximate standard deviation of basal area may be obtained when the diameter distribution is nearly normal, which avoids Σd^4 , as follows: From the moments of the normal curve, $\frac{\Sigma (r-M)^2}{N}$, it may be shown that $\Sigma d^2 = N(\sigma^2 + M^2)$ and $\Sigma d^4 = N(3\sigma^4 + 6M^2\sigma^2 + M^4)$; substituting these values in equation (6)

$$\begin{aligned} &\frac{k}{N} \sqrt{N \Sigma d^4 - (\Sigma d^2)^2} = \\ &k \sqrt{2\sigma^4 + 4M^2\sigma^2} = k \sqrt{2\sigma^2(\sigma^2 + 2M^2)} = \\ &k \sqrt{2 \left[\frac{\Sigma d^2}{N} - \left(\frac{\Sigma d}{N} \right)^2 \right] \left[\frac{\Sigma d^2}{N} - \left(\frac{\Sigma d}{N} \right)^2 + 2 \left(\frac{\Sigma d}{N} \right)^2 \right]} = \\ &k \sqrt{2 \left[\left(\frac{\Sigma d^2}{N} \right)^2 - \left(\frac{\Sigma d}{N} \right)^4 \right]} \end{aligned} \quad (7)$$

This approximation is useful because it uses values that are easily obtained, and even though in error it may serve to show that it is unnecessary to compute the true standard deviation.

A simple way to obtain the sums of the higher powers of d required in the above formulae is to list diameters by size in descending order and tabulate diameter, number, the product of these two, and three cumulative sums as follows:

d	n	nd	C_1	C_2	C_3
m	n_m	mn_m	mn_m	mn_m	mn_m
$m-1$	n_{m-1}	$(m-1)n_{m-1}$	mn_m + $(m-1)n_{m-1}$	$2mn_m$ + $(m-1)n_{m-1}$	$3mn_m + \dots$
$m-2$	n_{m-2}	$(m-2)n_{m-2}$	mn_m + $(m-1)n_{m-1}$ + $(m-2)n_{m-2}$	$3mn_m$ + $2(m-1)n_{m-1}$ + $(m-2)n_{m-2}$	$6mn_m + \dots$
3	n_3	$3n_3$			
2	n_2	$2n_2$			
1	n_1	n_1	S_0	S_1	S_2
Total N	S_0		S_1	S_2	S_3

Then $\Sigma d^2 = S_1$ and $\Sigma d^4 = 6S_3 - 6S_2 + S_1$.

These cumulative sums require that d be continuous from m to 1 inclusive, n_i may be zero, but its row will have entries in C_1 , C_2 , and C_3 . Note that this procedure provides a check, in that the last entry under the cumulative sums is the sum of the preceding column.

The product moment, or sum of cross products, is a statistic used in correlation analysis and regression equations. Where x is an associated variable—

$$\Sigma bx = k \Sigma d^2 x \quad (8)$$

Σbx may be readily obtained either by direct multiplying or more simply by the method of cumulative sums shown above. The sort is still on d , but, instead of showing nd , show Σx for each d value; then $\Sigma x = S_0$ and $\Sigma d^2 x = 2S_2 - S_1$.

The growth of a single tree in basal area is rarely needed, but its derivation aids in understanding what follows. Let Δd be growth in diameter and Δb be growth in basal area, then d and b are initial diameter and basal area, and $d + \Delta d$ and $b + \Delta b$ are diameter and basal area at any subsequent period.

Now—

$$b = kd^2$$

$$b + \Delta b = k(d + \Delta d)^2 = k(d^2 + 2d\Delta d + \Delta d^2)$$

and by subtraction—

$$\Delta b = k(2d\Delta d + \Delta d^2) \quad (9)$$

From which it readily follows that total basal-area growth,

$$\Sigma \Delta b = k(2\Sigma d\Delta d + \Sigma \Delta d^2) \quad (10)$$

and mean growth in basal area

$$\frac{\Sigma \Delta b}{N} = \frac{k}{N}(2\Sigma d\Delta d + \Sigma \Delta d^2) \quad (11)$$

There appears, unfortunately, to be no simple, direct means of obtaining $\sigma_{\Delta b}$ from d and Δd , but it may be computed in the usual way—

$$\sigma_{\Delta b} = \sqrt{\frac{\Sigma \Delta b^2}{N} - \left(\frac{\Sigma \Delta b}{N}\right)^2} \quad (12)$$

or with the usual correction factor for small samples. This formula requires the squaring, summing, and averaging of each individual Δb to obtain the first term, while the second may be secured from formula (11) above.

APPLICATION TO GROUPED DATA

With grouped data the practice usually recommended is to use the mid point of each class as the class value.⁴ Sheppard⁵ has shown that the average effect of grouping, using the mid point as the class value, is to introduce a positive bias of one-twelfth the square of the class interval in the average square when the class interval is constant, the distribution is continuous, and the class frequencies decrease very slowly in the tails (i. e., the frequency curve makes contact of high order with the abscissa). Since total or average basal area is a function of the sum of squares of diameter, it follows that with

grouped data a correction of $\frac{nhk^2}{12}$ or $\frac{kh^2}{12}$ when h is the class interval,

should be made. In the mean, where 1-inch classes are used, this amounts to only 0.0004545 . . . and it is of course correspondingly small in the total. It is evident that for all common purposes this correction is too small to be of any practical value. However, it should be noted that Sheppard's correction is meant to apply to the average result of grouping. In practice it is never worth while to obtain this average; but for certain distributions the results of a particular grouping may be as much as 10 percent different from values obtained without grouping. In general, grouped data will rarely produce the same result as would be obtained without grouping, but the discrepancies will be small and the grouping alone causes only a slight bias, if any. There is some loss in precision, but the statistics are much easier to compute.

But basal area as customarily obtained by grouping suffers a greater loss of precision than is necessary. The usual grouping of

⁴It might be supposed that the arithmetic class mean would provide a satisfactory class value, but it may be readily shown that this is too small by k times the sum of squares within classes.

⁵SHEPPARD, W. F. ON THE CALCULATION OF THE MOST PROBABLE VALUES OF FREQUENCY-CONSTANTS FOR DATA ARRANGED ACCORDING TO EQUIDISTANT DIVISIONS OF A SCALE. London Math. Soc. Proc. (1897-98) 29:353-380. 1898.

ON THE CALCULATION OF THE AVERAGE SQUARE, CUBE, ETC., OF A LARGE NUMBER OF MAGNITUDES. Jour. Roy. Statist. Soc. 60:698-703. 1897.

diameters puts the mid point of 1-inch classes at 0.05 greater than the even inch, and few basal-area tables show classes with diameter intervals of less than one-tenth of an inch. It thus becomes necessary to interpolate, and this is usually effected by using proportional parts. The error so introduced is small for individual trees, but it is always negative and may become appreciable in a sum.

Errors are also made by using the even inch as the mid point of the class, though it is usually 0.05 inch too small. This error may be readily corrected as shown below, but the correction is rarely made. Let A be the mid point of a class and a any deviation from A :

$$\Sigma b = k \Sigma d^2 = k \left(\Sigma A^2 - \frac{N h^2}{12} \right)$$

$k \Sigma A^2$ is obtained by using the true mid point.

$k \Sigma (A+a)^2$ is obtained by using any other point.

Ignoring k for the moment—

$$\Sigma (A+a)^2 = \Sigma A^2 + 2a \Sigma A + N a^2$$

whence

$$\Sigma A^2 = \Sigma (A+a)^2 - 2a \Sigma A - N a^2 = \Sigma (A+a)^2 - 2a \Sigma (A+a) + N a^2.$$

From this it is evident that no injury is done by using any arbitrary value desired for the class value in obtaining the sum of squares if the proper corrections are made. As a matter of fact, for ease of computation where the true mid point is some relatively awkward number, such as 5.05, the method of the assumed mid point is much simpler. Furthermore, since $\Sigma A' = \Sigma (A+a-a)$, it is very simple to obtain any of the higher moments needed even though a false class value is used:

$$\Sigma A = \Sigma (A+a) - N a \quad (13)$$

$$\Sigma A^2 = \Sigma (A+a)^2 - 2a \Sigma (A+a) + N a^2 \quad (14)$$

$$\Sigma A^3 = \Sigma (A+a)^3 - 3a \Sigma (A+a)^2 + 3a^2 \Sigma (A+a) - N a^3 \quad (15)$$

$$\Sigma A^4 = \Sigma (A+a)^4 - 4a \Sigma (A+a)^3 + 6a^2 \Sigma (A+a)^2 - 4a^3 \Sigma (A+a) + N a^4 \quad (16)$$

etc.

The proposed formula method uses formulae (13) to (16), with grouped data, to obtain $\Sigma A'$.

$$\Sigma A' = \Sigma d' + \text{Sheppard's correction.}$$

The formulae of ungrouped data, (3) to (12) inclusive, may then be used to obtain whatever statistics of basal area are needed.

A NUMERICAL EXAMPLE

The following example presents a summary of field data and the computations essential to the use of the preceding formulae.

From the original measurements (which are here excluded for the sake of brevity)—

$$N = 165$$

$$\Sigma d = 548.0$$

$$\Sigma d^2 = 1966.64$$

$$\Sigma d^4 = 31532.4704$$

$$\Sigma b = 10.724 \text{ (from table of basal area)}$$

$$k \Sigma d^2 = 10.726.$$

Table 1 shows the data grouped in the conventional manner with computations for two estimated values for each class.

TABLE 1.—*Computation of the sum of basal area by usual methods*

Diameter breast high interval (inches)	Trees (n)	Mid-point (A)	Approximate mid point (A+a)	Basal area by—			
				True value	A	A+a	Individual trees
1.6-2.5.....	Number 43	Inches 2.05	Inches 2	Sq. ft. 1.2244	Sq. ft. 0.989	Sq. ft. 0.946	Sq. ft. 1.216
2.6-3.5.....	66	3.05	3	3.4368	3.300	3.234	3.440
3.6-4.5.....	37	4.05	4	3.2952	3.293	3.219	3.700
4.6-5.5.....	17	5.05	5	2.3568	2.363	2.312	2.356
5.6-6.5.....	2	6.05	6	7.4128	7.398	7.392	7.412
Total.....	165			10.726	10.343	10.103	10.724

Table 2 shows the data grouped and computed by the formula method.

TABLE 2.—*Illustration of the formula method in obtaining the sum of basal areas*

Approximate mid point of diameter breast high (A+a) (inches)	Trees (n)	n (A+a)	C ₁	C ₂	Approximate mid point of diameter breast high (A+a) (inches)	Trees (n)	n (A+a)	C ₁	C ₂
6.....	Number 2	12	12	12	2.....	Number 43	86	529	1,326
5.....	17	85	97	109	1.....			529	1,855
4.....	37	148	245	354					
3.....	66	198	443	797	Total.....	165	529	1,855	

Σd^2 is approximately $\Sigma(A+a)^2 - 2a \Sigma(A+a) + Na^2$ or $1855 - 2(-0.05)(529) + (165)(-0.05)^2 = 1908.3125$. $\Sigma b = k \Sigma d^2$ or approximately $1908.3125 k = 10.408$. Note that Σb without correction (14) is $1855k$ or 10.117.

Of the six values for the sum of basal area, the one most likely to be right is 10.726 obtained from $k \Sigma d^2$. That obtained by summing the tabular values for individual trees checks as closely as can be expected.

The result of using the true mid points is contrary to what would be expected for this method on the average, but it should be remembered that this is a particular sample and the generalization applies to the averages of all possible groupings. This does not imply or even suggest that there is anything wrong with the sample, as certain other unexpected results might. In this particular example the 10 possible groupings of diameter were computed and total basal area varied from 9.520 to 12.255 and averaged 11.039. Sheppard's correction of -0.075 helped, but at 10.964 is still 2 percent too large.

The employment of the even inch as the class mid point yields, as was expected, a result that is too small. When the correction 53.3125 is added to 1,855 it precisely checks 1,908.3215, as it should.

The discrepancies in total basal area as obtained from the table and from summing the squares and correcting by the factor k are due to the lack of precision of the table, as is shown by the following comparison (table 3).

TABLE 3.—*Comparison of errors introduced by using too few decimal places*

A (inches)	Table	kd^2	$A+a$ (inches)	Table	kd^2
	<i>Sq. ft.</i>	<i>Sq. ft.</i>		<i>Sq. ft.</i>	<i>Sq. ft.</i>
2.05	0.023	0.02292	2	0.022	0.02182
3.05	.050	.05074	3	.049	.04909
4.05	.089	.08946	4	.087	.08726
5.05	.139	.13909	5	.136	.13635
6.05	.199	.19963	6	.196	.19634

These differences, although small for a particular entry, become increasingly important as the n of the class increases and even without a tendency to be conservative introduce a bias toward too small a value when using the basal-area table with any sort of class values. Note that 4 of the 5 entries in each group have a larger value for kd^2 than for the table value. (Interpolation for the values of A was made assuming a straight line, which is the common practice, although it is admittedly erroneous.) It thus becomes apparent that to obtain precision in the sum of basal area by means of grouped data equal to that obtained from ungrouped data using a table which shows diameter to 1 decimal place and basal area to 3 decimal places, it is essential to have a table showing diameter to 2 and basal area to 5 decimal places, as well as to correct for the errors of grouping, or to use other means than a table.

The other statistics of basal area as computed by the formulae are presented in table 4:

 TABLE 4 *Comparison of other statistics of basal area as ascertained by various methods*

source	Average basal area	Diameter breast high of average basal area	$\sqrt{\frac{\sum d^2}{N}}$	Standard deviation σ_b	Approximate stand- ard deviation σ_b
	<i>Sq. ft.</i>	<i>Inches</i>	<i>Inches</i>	<i>Sq. ft.</i>	<i>Sq. ft.</i>
Table and A	0.0627	3.4			
Table and $A+a$0612	3.3			
Individual d0650	3.45	3.45	0.03819	0.03483
Table and individual d0650	3.45			
Sum of A^20631	3.4	3.40		.03565
Sum of $(A+a)^2$0613	3.3	3.35		.03512

The points of interest here are already discussed for sums and will differ only in their values, not in their relation to one another. The σ'_b obtained from any of the four methods shows essential agreement with σ_b , but there is a discrepancy in the wrong direction which is too large when there is any doubt of the result of the test for which it is used.

The formulae of cross products and growth, though equally important, are not illustrated because to do so would require another example and the arithmetic involves no new principles.

SUMMARY

Statistics of basal area may be readily obtained by the "formula method" without using a table of basal area. No one method will, however, meet all situations; the best way for each particular case depends on the precision required, the size of the sample, and the mechanical aids available. For very small samples, when only simple statistics such as the sum and mean are wanted, the basal-area table is the easiest way of obtaining them. For more complex statistics from relatively small samples, the most efficient procedure is to use formulae (3), (4), (5), (6), (8), (10), and (12), as needed, without grouping. For large samples, the easiest way is to group, using intervals of even inches, and make the corrections indicated by formulae (13) to (16), remembering that precision is always lost by grouping.

The formulae presented are reassembled here for ready reference:

Total basal area:

$$k\Sigma d^2 \quad (3)$$

Average basal area:

$$\frac{k}{N}\Sigma d^2 \quad (4)$$

Diameter of average basal area:

$$\sqrt{\frac{\Sigma d^2}{N}} \quad (5)$$

Standard deviation of basal area:

$$\frac{k}{N}\sqrt{N\Sigma d^4 - (\Sigma d^2)^2} \quad (6)$$

or

$$\frac{k}{N}\sqrt{\frac{N}{N-1}}\sqrt{N\Sigma d^4 - (\Sigma d^2)^2}$$

Approximate standard deviation of basal area:

$$k\sqrt{2}\sqrt{\left(\frac{\Sigma d^2}{N}\right)^2 - \left(\frac{\Sigma d}{N}\right)^4} \quad (7)$$

or

$$k\sqrt{\frac{2N}{N-1}}\sqrt{\left(\frac{\Sigma d^2}{N}\right)^2 - \left(\frac{\Sigma d}{N}\right)^4}$$

Product moment:

$$\Sigma bx = k\Sigma d^2 x \quad (8)$$

Basal area growth:

$$\Delta b = k(2d\Delta d + \Delta d^2) \quad (9)$$

Total basal-area growth:

$$\Sigma \Delta b = k(2\Sigma d\Delta d + \Sigma \Delta d^2) \quad (10)$$

Standard deviation of basal area growth:

$$\sqrt{\frac{\Sigma \Delta b^2}{N} - \left(\frac{\Sigma \Delta b}{N}\right)^2} \quad (12)$$

or

$$\sqrt{\frac{N}{N-1}} \sqrt{\frac{\Sigma \Delta b^2}{N} - \left(\frac{\Sigma \Delta b}{N}\right)^2}$$

Corrections for using a false mid point:

Sum of first power:

$$Na \quad (13)$$

Sum of second power:

$$2a\Sigma(A+a) - Na^2 \quad (14)$$

Sum of third power:

$$3a\Sigma(A+a)^2 - 3a^2\Sigma(A+a) + Na^3 \quad (15)$$

Sum of fourth power:

$$4a\Sigma(A+a)^3 - 6a^2\Sigma(A+a)^2 + 4a^3\Sigma(A+a) - Na^4 \quad (16)$$

THE EFFECT OF ONE AND OF TWO SEEDLING LETHALS IN THE HETEROZYGOUS CONDITION ON BARLEY DEVELOPMENT¹

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INTRODUCTION

The effect of recessive characters in the heterozygous condition may have some economic importance in yield of cereal crops. The studies reported in this paper were made to determine the effect of 1 or of 2 lethal factors in the heterozygous condition on the growth of barley plants.

LITERATURE REVIEW

A summary of the recent literature on the effect of lethal characters on the growth of various crops is given in a previous publication by Robertson.²

A previous paper reported that the factor pair $A_c a_c$ for white versus green seedlings in Colseess I, the factor pair $X_h r_h$ for yellow versus green seedlings in Black Hull-less, and the factor pair $A_h a_h$ for green versus white seedlings in Hanna, when in the heterozygous condition, had no effect on the following mature-plant characters: Number of culms, average length of culm, average length of head, number of grains per plant, and total grain yield per plant. With the factor pair $A_c a_c$ for white versus green seedlings in Canada Thorpe was associated an endosperm effect. In mature seeds borne on plants carrying the double-recessive factor pair $a_c a_c$, this endosperm effect was evident and reduced the yield. However the other plant characters were not affected.

MATERIAL AND METHODS

Colseess I³ produced a white seedling in a monohybrid ratio. This seedling developed for about 10 days and then died.

Colseess II³ produced a white seedling in a monohybrid ratio. This seedling behaved similarly to Colseess I, but differed from Colseess I genetically.

Colseess IV³ produced a yellow seedling in a monohybrid ratio. This seedling grew under field conditions (low temperature) for about 10 days and then died. It has previously been reported as being linked with the factor pair $A_c a_c$ found in Colseess I. Crosses were made between Colseess I and Colseess IV in order to study the effect of the lethals in the heterozygous condition on some mature-plant characters. These characters were number of culms per plant, average length of culm per plant, average length of head per plant, number of grains per plant, and total weight of grain per plant. The segregation of the F_2 and F_3 plants again showed linkage.

¹ Received for publication Apr. 18, 1935; issued December 1935.

² ROBERTSON, D. W. THE EFFECT OF A LETHAL IN THE HETEROZYGOUS CONDITION ON BARLEY DEVELOPMENT. *Colo. Agr. Expt. Sta. Tech. Bull.* 1, 12 pp., illus. December 1932.

³ ROBERTSON, D. W. LINKAGE STUDIES IN BARLEY. *Genetics* 14:1-36 1929.

Crosses were also made between Colseess II and Colseess IV. The numbers found in the F_2 seedlings are shown in table 1.

The data indicate independent inheritance of the factor pairs $A_{c_3}a_{c_3}$ and X_{c_c} . To further test the interrelationship of the factor pairs, the green F_2 plants were grown in F_3 and seedling counts made. The genetic constitution of the green F_2 plants as determined by the F_3 seedling behavior is given in table 2.

TABLE 1.—Segregation of seedling color in the F_2 of a cross between Colseess II ($A_{c_3}a_{c_3}$) and Colseess IV (X_{c_c})

Item	Green	Yellow	White
Observed number.....	1,025	335	431
Calculated segregation 9 : 3 : 4.....	1,007.44	335.81	447.75
	$\chi^2 = 0.9347$ P , very large		

TABLE 2.—Genetic constitution of the F_2 green plants as determined by F_3 seedling numbers

Item	Indicated genotypes			
	$A_{c_3}A_{c_3}X_{c_c}X_{c_c}$	$A_{c_3}a_{c_3}X_{c_c}X_{c_c}$	$A_{c_3}A_{c_3}X_{c_c}x_{c_c}$	$A_{c_3}a_{c_3}X_{c_c}x_{c_c}$
	Number	Number	Number	Number
Observed number.....	63	124	140	256
Calculated segregation 1 : 2 : 2 : 4.....	64.78	129.55	129.55	259.12
	$\chi^2 = 1.1672$ $P = 0.7630$			

The F_3 data give a good fit to a calculated 1 : 2 : 2 : 4 ratio for independent inheritance of the factor pairs $A_{c_3}a_{c_3}$ and X_{c_c} .

The F_1 seeds were sown in 18-foot rows 1 foot apart, and spaced about 3 inches apart in the rows. Because of the presence of lethal seedlings in the F_2 population, the plants were harvested in two lots, the competitive plants, those growing adjacent to other plants in the rows being harvested separately. The remaining plants, the non-competitive plants, with blank spaces adjacent to them, were also harvested separately. The presence of blanks in adjacent rows was not considered. After the growth data were obtained, 50 seeds from each F_2 plant were sown in moist sand, and the F_2 genetic constitution of the plants determined from F_3 seedling numbers.

EXPERIMENTAL RESULTS

A comparison was made of both the pure green ($A_{c_3}A_{c_3}$) and the heterozygous green and white ($A_{c_3}a_{c_3}$) F_2 plants in both the competitive and the noncompetitive groups. The plants were harvested and the measurements made during the winter of 1934-35. Fifty seeds from each plant were grown in the greenhouse to determine the genetic constitution of the plants for the seedling factors. The plants were then grouped according to their genetic constitution, and the data

analyzed. The results obtained from the green plants of the genetic constitution $A_{c_3}A_{c_3}$ and from the green plants heterozygous for the genetic factor pair $A_{c_3}a_{c_3}$ are given in table 3.

In the competitive plants no significant differences were found between the green and heterozygous plants. In the noncompetitive plants the same trend was found. The only significant difference was in the average length of culm, which, however, was in favor of the heterozygous plants. Since the trend was the same in both competitive and noncompetitive plants, only competitive plants were used in the study of the other factor combinations.

TABLE 3.—Measurements of plant characters in Colless II plants grown at Fort Collins, Colo.

Characters studied	Segregation	Plants	Mean	Standard error of mean	Difference	Standard error of a difference	1)/S. E. of difference	S. E. in percentage of mean
		Number						Percent
Competitive plants:								
Culms per plant, number.	(Green.....	150	5.1533	0.1391	} +0.1979	0.1956	1.0118	{ 2.6902
	(Heterozygous..	168	5.3512	.1375				
Average length of culm, centimeters.	(Green.....	150	62.5933	.4709	} +.4543	.6559	.6296	{ .7523
	(Heterozygous..	168	63.0476	.4566				
Average length of head, centimeters.	(Green.....	150	6.8533	.0532	} -.1003	.0745	1.3463	{ .7763
	(Heterozygous..	168	6.7530	.0521				
Total grains, number.	(Green.....	150	210.3300	5.8412	} -4.97	8.0920	.6142	{ 2.7772
	(Heterozygous..	168	205.3600	5.6002				
Total weight of grain, grams.	(Green.....	150	6.3200	.1889	} -.1682	.2484	6771	{ 2.9889
	(Heterozygous..	168	6.1518	.1613				
Noncompetitive plants:								
Culms per plant, number.	(Green.....	115	5.8609	.1905	} +1455	.2579	5642	{ 3.2504
	(Heterozygous..	157	6.0084	.1738				
Average length of culm, centimeters.	(Green.....	115	61.5000	.5543	} +1.5032	7446	2.0188	{ .9013
	(Heterozygous..	157	63.0032	.4972				
Average length of head, centimeters.	(Green.....	115	7.0239	.0660	} -.0287	.0867	3310	{ .8396
	(Heterozygous..	157	6.9952	.0562				
Total grains, number.	(Green.....	115	240.2200	7.9739	} +7.07	10.8828	.6497	{ 3.3194
	(Heterozygous..	157	247.2900	7.4063				
Total weight of grain, grams.	(Green.....	115	7.6630	.2507	} +.2813	.3474	.8097	{ 3.2716
	(Heterozygous..	157	7.9443	.2405				

GREEN VERSUS YELLOW SEEDLINGS ($X_c x_c$)

Crosses involving Colless IV ($X_c x_c$) were used in the study of green versus yellow seedlings. Table 4 gives the summarized data for the characters studied. The average length of head is the only one of the characters studied in the plants heterozygous for green versus yellow seedling color significantly different from the same characters in the homozygous green plants.

GREEN VERSUS WHITE SEEDLINGS ($A_c a_c$)

Heterozygous green plants ($A_c a_c$) and homozygous green plants ($A_c A_c$) were compared in crosses involving Colless I. The data obtained from this study are also tabulated in table 4. None of the characters examined in the heterozygous plants differs statistically significantly from the same characters in the homozygous green plants. This indicates that the seedling factor pair $A_c a_c$, which produced green and white seedlings in Colless I, is not detrimental to the growth of green plants having the genetic constitution $A_c a_c$.

TABLE 4.—Measurements of plant characters in crosses involving Colseess IV and Colseess I (competitive plants), grown at Fort Collins, Colo.

Characters studied	Segregation	Plants	Mean	Stand- ard error of mean	Differ- ence	Stand- ard error of a differ- ence	1)/S. E. of differ- ence	S. E. in per- centage of mean
Colseess IV:		Number						Percent
Culms per plant, num- ber.	Green.....	104	5.7308	0.1856	-0.1600	0.2306	0.6938	3.2386
	Heterozygous.	219	5.5708	.1308				2.4557
Average length of culm, centimeters.	Green.....	104	62.3846	.6201	+.8323	.7261	1.1463	.9940
	Heterozygous.	219	63.2169	.3777				.5975
Average length of head, centimeters.	Green.....	104	6.2885	.0669	+.4592	.0841	5.4002	1.0638
	Heterozygous.	219	6.7477	.0510				.7558
Total grains, number.	Green.....	104	207.2100	7.3518	+10.2558	9.0260	1.1363	3.5480
	Heterozygous.	219	217.4700	5.2364				2.4079
Total weight of grain, grams	Green.....	104	6.7500	.2280	-.0068	.2841	.0239	3.3778
	Heterozygous	219	6.7432	.1696				2.5151
Colseess I:								
Culms per plant, num- ber.	Green.....	104	5.7308	.1856	+0.2317	.2269	1.0212	3.2386
	Heterozygous	160	5.9623	.1305				2.1887
Average length of culm, centimeters.	Green.....	104	62.3846	.6201	+1.6904	1.7937	2.1298	.9940
	Heterozygous	160	64.0750	.4954				.7732
Average length of head, centimeters	Green.....	104	6.2885	.0669	+1.4900	.0872	.7087	1.0638
	Heterozygous	160	6.4375	.0500				.8099
Total grains, number.	Green.....	104	207.2100	7.3518	+9.04	9.1995	9827	3.5480
	Heterozygous	160	216.2500	5.5300				2.5872
Total weight of grain, grams	Green.....	104	6.7500	.2280	+1.4338	.2814	.5100	3.3778
	Heterozygous.	160	6.8938	.1649				2.3920

THE EFFECT OF TWO LETHAL FACTORS

The effect of two lethal factor pairs when in the heterozygous condition on the growth of plants was determined in families from a cross between Colseess II and Colseess IV. The factor pairs involved in this cross are $A_{c_3}a_{c_3}$ and $X_c x_c$. The data obtained from plants heterozygous for both factor pairs are given in table 5.

TABLE 5.—Measurements of plant characters in competitive plants heterozygous for two lethal-factor pairs $A_{c_3}a_{c_3}$ and $X_c x_c$, or for the two linked-factor pairs $A_c a_c$ and $X_c x_c$

Characters studied	Segregation	Plants	Mean	Stand- ard error of mean	Differ- ence	Stand- ard error of a differ- ence	1)/S. E. of differ- ence	S. E. in per- centage of mean
$A_{c_3}a_{c_3}$ and $X_c x_c$:		Number						Percent
Culms per plant, num- ber.	Green.....	150	5.1533	0.1391	+0.3632	0.2394	1.5171	2.6992
	Heterozygous.	91	5.5165	.1949				3.5330
Average length of culm, centimeter.	Green.....	150	62.5933	.4709	-2.2362	.7451	3.0012	.7523
	Heterozygous.	91	60.3571	.5774				.9566
Average length of head, centimeters.	Green.....	150	6.8533	.0532	-1.088	.0981	1.1091	.7763
	Heterozygous.	91	6.7445	.0824				1.2217
Total grains, number.	Green.....	150	210.3300	5.8412	-1.54	10.5705	.1457	2.7772
	Heterozygous.	91	208.7900	8.8100				4.2196
Total weight of grain, grams.	Green.....	150	6.3200	.1889	+3.146	.3246	9692	2.9889
	Heterozygous.	91	6.6346	.2640				3.9791
$A_c a_c$ and $X_c x_c$:								
Culms per plant, num- ber.	Green.....	104	5.7308	.1856	+.4134	.2764	1.4957	3.2386
	Heterozygous.	104	6.1442	.2048				3.3332
Average length of culm, centimeters.	Green.....	104	62.3846	.6201	+.9231	.8978	1.0282	0.9940
	Heterozygous.	104	63.3077	.6493				1.0256
Average length of head, centimeters.	Green.....	104	6.2885	.0669	+.4327	.0989	4.3751	1.0638
	Heterozygous.	104	6.7212	.0728				1.0831
Total grains, number.	Green.....	104	207.2100	7.3518	+31.73	11.3440	2.7971	3.5480
	Heterozygous.	104	238.9400	8.6390				3.6156
Weight of grain per plant, grams.	Green.....	104	6.7500	.2280	+1.0000	.3483	2.8711	3.3778
	Heterozygous.	104	7.7500	.2632				3.3961

With the exception of the average length of culm, none of the differences between the characters studied in homozygous green plants and plants heterozygous for both factor pairs is significant. The green plants seem to have a higher average length of culm than the heterozygous plants. However, the general indications are that two lethals in the heterozygous condition are not detrimental to the growth of plants as indicated by the measurements of the number of culms, average length of head, total number of grains, and total grain weight.

A study of the linked genes in Colless IV and Colless I was made in the plants heterozygous for both of the factor pairs $X_c x_c$ and $A_c a_c$. The data obtained from this study are also given in table 5.

In the study of the above-mentioned characters, it was found that all of the plants surviving in F_2 were heterozygous for both factor pairs. As the linkage of the factor pairs $A_c a_c$ and $X_c x_c$ shows less than 4 percent crossing over,⁴ one would not expect any green plants in the small number of competitive plants studied. This made it impossible to compare the heterozygous plants with homozygous green plants of the same family. However, green plants from the families segregating for the single factor pairs $X_c x_c$ and $A_c a_c$ were used. The data in table 5 show significant differences in favor of the heterozygous plants for the following characters: Average length of head, total number of grains per plant, and total weight of grain per plant. The variability of the green and heterozygous plants is uniform for the same plant characters. This is indicated in the column headed S. E. in percentage of mean. A similar procedure was used in studying the relationship in the nonlinked factor pairs $X_c x_c$ and $A_{c_3} a_{c_3}$, except that some green plants from the families segregating for both factor pairs were used. The discrepancy can hardly be explained on the basis of blank spaces in the adjacent rows since the same ratio of 9 green to 7 lethal seedlings was obtained in both families. The results indicate, as in the case of the nonlinked genes, that there is no detrimental effect of two lethal genes in the heterozygous condition on the growth characters studied in Colless.

DISCUSSION

The data presented show less difference between the competitive and noncompetitive plants than was found in the previous study.⁵ The same lack of detrimental effect is evident in both cases for the factor pair $A_c a_c$ found in Colless I.

The apparent difference in favor of the plants heterozygous for both the factor pairs $A_c a_c$ and $X_c x_c$ cannot be explained on the basis of the presence of lessened competition due to blank spaces in the adjacent rows since this condition was also present in the plants used for the study of the effect of the factor pairs $A_{c_3} a_{c_3}$ and $X_c x_c$, which are not linked. In the latter case no significant difference was found between the green plants of the genetic constitution $(A_{c_3} A_{c_3}) (X_c X_c)$ and the above-mentioned green F_2 plants of the genetic constitution $(A_{c_3} a_{c_3}) (X_c x_c)$. If the difference had been due to blank spaces in the adjacent rows the same difference should have been obtained in both families studied since the percentage of blank spaces was the same.

⁴ ROBERTSON, D. W. See footnote 3.

⁵ ROBERTSON, D. W. See footnote 2.

There is a possibility that the difference may be due to the recombining of dominant-growth genes linked with the lethal-factor pairs in Colseess I and Colseess IV.

SUMMARY

The effects of the lethal seedling-factor pairs $A_c a_c$, found in Colseess I, $A_{c_3} a_{c_3}$, found in Colseess II, and $X_c x_c$, found in Colseess IV, were studied as single heterozygotes in different plants and in combinations of double heterozygotes in different plants on the following mature-plant characters: Number of culms per plant, average length of culm per plant, average length of head per plant, total number of grains per plant, and total grain weight per plant.

Colseess I ($A_c a_c$) produced green and white seedlings in a 3 : 1 ratio. Colseess II ($A_{c_3} a_{c_3}$) also produced green and white seedlings in a 3 : 1 ratio, but is different genetical y from Colseess I. Colseess IV ($X_c x_c$) produced green and yellow seedlings in a 3 : 1 ratio and is linked to the factor pair ($A_c a_c$) in Colseess I.

No detrimental effects were found when plants of Colseess I, Colseess II, and Colseess IV in the heterozygous condition were compared with homozygous green plants of the same families.

No detrimental effects were found in the characters studied when plants containing the factor pairs ($X_c x_c$) ($A_{c_3} a_{c_3}$), double heterozygotes, were compared with pure green plants of the same families.

A similar lack of detrimental effect on the characters studied was found when plants containing the linked-factor pairs ($X_c x_c$) ($A_c a_c$), double heterozygotes, were compared with related green plants from the same cross.

SOIL-TEMPERATURE STUDIES ON FLORIDA CIGAR-WRAPPER TOBACCO¹

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INTRODUCTION

Numerous studies have been made to determine the effect of temperature on the growth of certain varieties of tobacco (*Nicotiana tabacum* L.), and on the development of certain diseases of this host. Several workers have studied the relation between soil temperature and germination of tobacco seed. Kincaid (8)² found that the cardinal temperatures for the germination of Florida cigar-wrapper tobacco seeds are approximately 10°, 24°, and 34° C.

The extent to which the growth of tobacco plants is affected by soil temperature apparently has received little attention. Johnson and Hartman (5) reported that white burley plants grew very little at temperatures below 13° C., best at 29° or 31°, and poorly at 40°; they noted that at the optimum temperature the plants grew low and stocky with broad but rather pointed leaves, while at temperatures near the maximum the plants were tall and spindly with short and rounded leaves. Godfrey (2), who also experimented with white burley tobacco, reported that there was a uniform increase in growth at temperatures from 10° to 25°, and some growth at 38°, the highest temperature which he used.

The effect of soil temperature on the development of certain soil-borne diseases of tobacco, especially black root rot, has received considerable attention, as is shown in a review by Jones, Johnson, and Dickson (7). These writers have also presented a valuable discussion of the relation of soil temperature to plant disease, making further discussion of this subject unnecessary here.

Tisdale and Kelley (10) made observations on the relationship between soil temperature and the development of black shank caused by *Phytophthora parasitica* var. *nicotianae* Tucker. They reported that plants transplanted early remained free of the disease for a few weeks, until the mean daily temperature of the soil reached about 20° C., and that the temperature during the remainder of the season never rose too high for infection.

The cardinal temperatures for the growth on culture media of a strain of the black shank *Phytophthora* isolated at the North Florida Experiment Station are approximately 8°, 30°, and 36° C., according to data reported by Tisdale and Kelley (10) and Tucker (11).

Black shank is the most important disease of tobacco in the district of northern Florida and southwestern Georgia where cigar-wrapper

¹ Received for publication May 13, 1935; issued December 1935.

² Reference is made by number (italic) to Literature Cited, p. 449.

tobacco is grown under shade. This disease was first identified in the United States by Tisdale (9) in 1922, and by breeding and selection he produced varieties of cigar-wrapper tobacco highly resistant to it.

The object of the investigation reported below was to determine the cardinal soil temperatures both for the development of the black shank disease and for the growth of Florida cigar-wrapper tobacco plants. Some observations were made on the relation of soil temperature to the development of black shank in the field.

APPARATUS, METHODS, AND MATERIALS

The apparatus used in these studies for the control of soil temperature was similar to that described by Camp and Walker (1). It consisted of eight compartments filled with water. Each compartment accommodated eight cans of soil, and the level of the water outside the cans was about the same as the level of the soil inside the

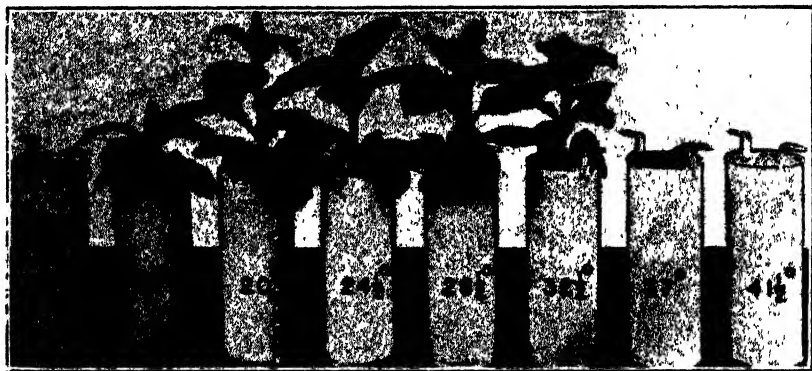


FIGURE 1. --Effect of constant soil temperatures (indicated on the cans) on the growth of tobacco plants of the Round Tip variety, see table 1, trial 2.

cans. An agitator in each compartment kept the water in continuous circulation. The compartment at one end of the apparatus was cooled by means of a thermostatically controlled refrigerator, and the compartment at the other end was heated by means of thermostatically controlled electric heaters of the immersion type. The other six compartments adjusted themselves to a series of intermediate temperatures, differing by 3° to 5°.

The cans (fig. 1), made of galvanized iron, were 8 inches in diameter and 22 inches deep, and had a capacity of about 4 gallons of soil. They were brought to equal tare by the addition of coarse gravel, and the same weight of moist soil was added to each.

The soil was a fine sandy loam having a pH value of about 5.2. The fertilizer used was composed of 100 pounds of cottonseed meal, 10 pounds of precipitated bone, and 15 pounds of sulphate of potash, and analyzed 8 percent NH_3 , 8 percent P_2O_5 , and 5 percent K_2O . It was applied at the rate of 5 ounces per 4-gallon can. Before the fertilized soil was used in these experiments, it was heated in an oven for about 6 hours at 60° to 70° C., or for about 2 hours at 80° to 90°, to destroy the black shank fungus and root knot nematodes.

The water-holding capacity of the soil was determined by the Hilgard (4) method, and the moisture content by drying samples in an oven at 105°. The initial water content of the soil in the different trials varied from 14 to 19 percent of the dry weight, or from 32 to 40 percent of the water-holding capacity. In the first two trials, the water content of the soil in the coldest compartment increased to about 25 percent of the dry weight as a result of the condensation of atmospheric moisture on the cold inner surface of the cans. However, the appearance of the plants in the cans was normal, and the difference in the moisture content of the soil was not believed to be of much importance. A 1-inch layer of coarsely granulated cork was added to the top of the soil after the plants had been transplanted, for the purpose of insulation.

The temperatures observed in the soil at a depth of 4 inches seldom varied more than 1° above or below the average temperatures reported in the tables. The mean daily temperature of the greenhouse in which the apparatus was located ranged from 23° C. in one trial conducted in midwinter to 29° C. in another trial conducted in early summer, and the daily range was generally between 10° and 15°. Conditions in the greenhouse during all trials were favorable for the growth of tobacco plants. The cans were weighed once a week, and sufficient tap water was added to the soil once or twice a week to restore them to their original weight, but no allowance was made for the weight of the plants.

The plants used in these experiments were of the variety No. 301, which is resistant, and Round Tip,³ which is highly susceptible to black shank. No. 301 was developed at this station by Tisdale and Round Tip was developed in Connecticut by Hayes, East, and Beinhart and by Jones (3, 6). Seed plants had been self-pollinated for several successive generations, and the seeds used were probably pure lines. The seedlings were grown in flats of sterilized soil. They were carefully selected for uniformity of size and color, and one seedling was transplanted to each can. The height of the plants at the time of transplanting was about 1½ inches.

THE CARDINAL SOIL TEMPERATURES FOR THE GROWTH OF TRANSPLANTED TOBACCO SEEDLINGS

Several trials were conducted to determine the cardinal soil temperatures for the growth of cigar-wrapper tobacco plants for a period of a few weeks after transplanting. These temperatures may be defined as follows: Minimum, the lowest temperature at which growth occurs; optimum, the temperature at which the most growth occurs; maximum, the highest temperature at which growth occurs.

A few days after the seedlings had been transplanted, the cans were placed in the tanks and brought to the various constant temperatures. After a certain period of growth, observations were made on the plants as follows: (1) Height from the soil to the terminal bud, (2) length of the longest leaf, (3) number of leaves longer than 1

³ Both of these varieties may be classified under type 62,⁴ which comprises southern shade tobacco grown for cigar wrappers.

⁴ UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF AGRICULTURAL ECONOMICS. STANDARD GRADES FOR SOUTHERN SHADE TOBACCO (U. S. TYPE 62). 15 pp. 1933. [Micrographed.]

inch, and (4) the green weight of the plants. Measurements of green weight were made only on the plants of No. 301; the Round Tip plants were used for the studies on black shank.

A preliminary trial was conducted with Round Tip plants to obtain an indication of the limits of temperature for growth. Because of

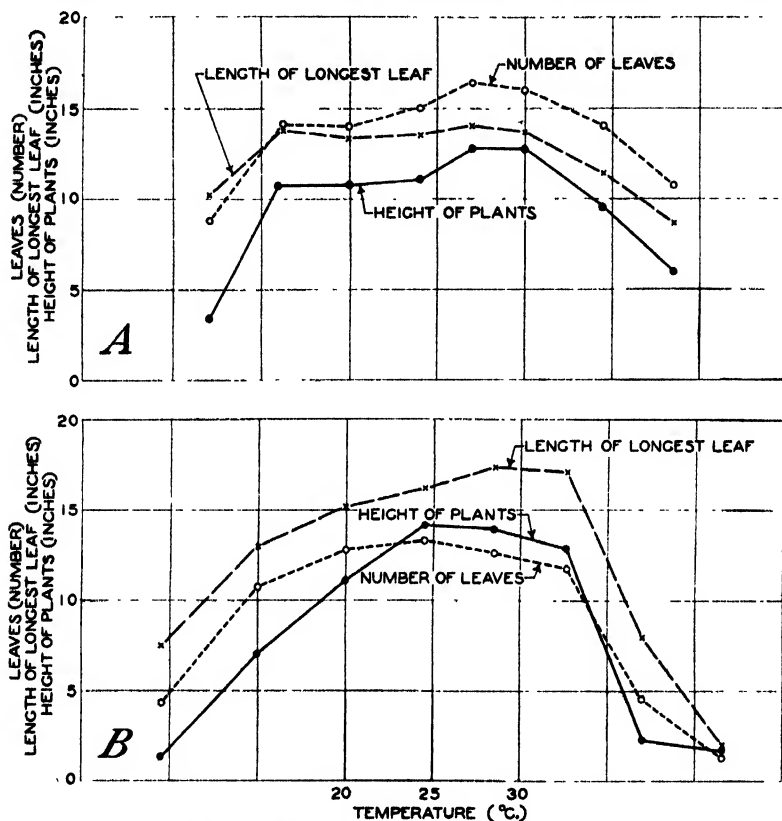


FIGURE 2.—Effect of various constant soil temperatures on the growth of tobacco plants of the Round Tip variety: A, Trial 1; B, trial 2.

mechanical difficulties incidental to the adjustment of the refrigerator, the control at the cold end of the apparatus was poor. However, it was evident from the results that the minimum was slightly below the lowest temperature used, 16° C. and below, and that the maximum was approximately 40°.

Three other trials with Round Tip and two trials with no. 301 plants were conducted. The average measurements of the plants in these five trials are given in table 1. The growth of Round Tip plants in trial 2 is shown in figure 1; the plants selected for the photograph were those which best illustrated the average measurements of

the plants grown in the respective compartments. The growth of the plants in trials 1, 2, 4, and 5 is shown graphically in figures 2 and 3.

These results indicate that the minimum temperature for growth of transplanted seedlings of both Round Tip and No. 301 is about 9° C.,

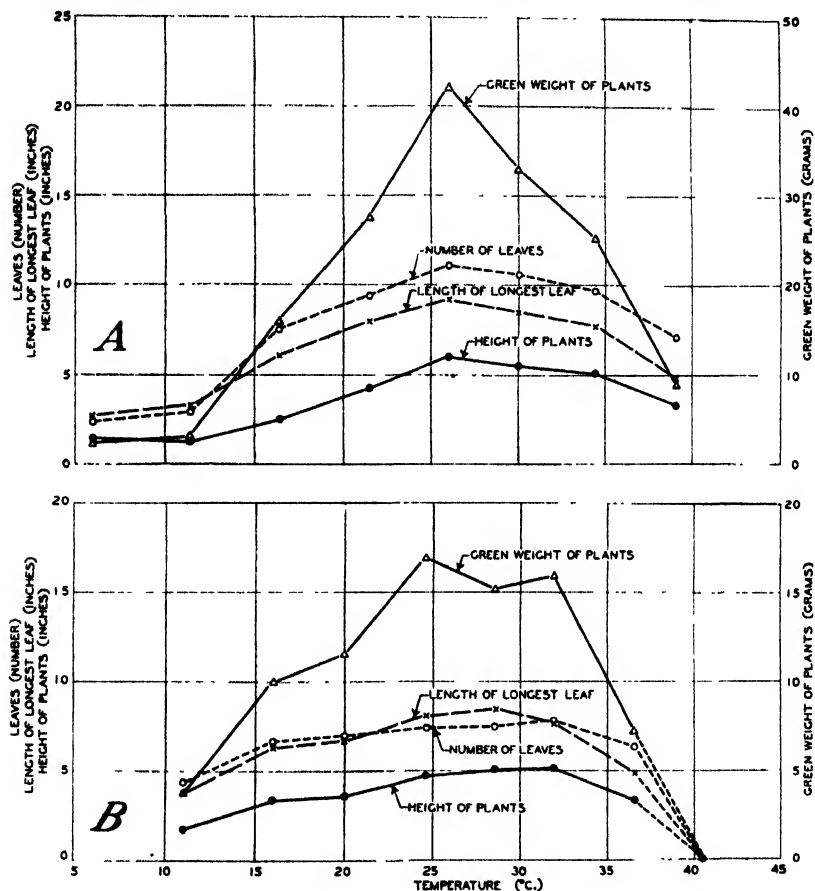


FIGURE 3.—Effect of various constant soil temperatures on the growth of tobacco plants of variety No. 301: A, Trial 4; B, trial 5.

and the maximum about 40°. The optimum was not very closely defined, because the best growth, as indicated by the measurements, was distributed over a range of temperatures from 24½° to 32°.

The minimum for the growth of transplanted seedlings corresponds closely to the minimum for the germination of seeds of No. 301 (8) but the optimum and maximum are somewhat higher.

TABLE 1.—Effect of various constant soil temperatures on the growth of tobacco plants of the Round Tip and No. 301 varieties

Trial no.	Variety	Period of observation	Soil temperature	Plants	Average height of plants	Average length of longest leaf	Average number of leaves	Average green weight per plant
		Days	° C.	Number	Inches	Inches		Grams
1	Round Tip	47	12	7	3.3	10.1	8.7	-----
			16	16	10.7	13.9	14.0	-----
			20	16	10.7	13.3	14.0	-----
			24	7	11.0	13.5	15.0	-----
			27	7	12.8	14.0	16.4	-----
			30	7	12.8	13.7	16.0	-----
			34 ¹ / ₂	7	9.6	11.3	14.0	-----
			38 ¹ / ₂	7	6.0	8.6	10.7	-----
			41 ¹ / ₂	7	1.3	7.3	4.4	-----
			45	7	7.1	13.0	10.8	-----
2	do.	32	20	7	11.1	15.1	12.8	-----
			24 ¹ / ₂	7	14.0	16.1	13.3	-----
			28 ¹ / ₂	7	13.9	17.3	12.7	-----
			32 ¹ / ₂	7	12.8	17.1	11.8	-----
			37	7	2.3	7.9	4.6	-----
			41 ¹ / ₂	7	1.8	1.9	1.4	-----
			45 ¹ / ₂	1	9.5	8.5	11.0	-----
			16	1	17.0	12.5	17.0	-----
			20	1	18.5	13.5	18.0	-----
			24 ¹ / ₂	1	18.5	14.5	17.0	-----
3	do.	49	28	1	21.5	14.0	20.0	-----
			31 ¹ / ₂	1	21.5	13.0	18.0	-----
			35	1	13.5	10.5	13.0	-----
			39 ¹ / ₂	1	4.0	4.5	5.0	-----
			46	7	1.5	2.8	2.4	2.4
			49 ¹ / ₂	7	1.3	3.3	3.0	2.9
			53 ¹ / ₂	7	2.7	6.1	7.7	16.1
			57 ¹ / ₂	7	4.3	8.1	9.7	27.6
			60	7	6.1	9.5	11.1	42.1
			63	7	5.6	8.7	10.7	33.3
4	No. 301	31	30	7	5.1	7.9	9.9	25.7
			34 ¹ / ₂	7	3.4	4.9	7.1	9.3
			39	7	1.8	3.9	4.4	3.9
			41	8	3.3	6.4	6.6	10.0
			46	7	3.6	6.8	7.0	11.7
			50	7	4.8	8.0	7.6	17.0
			54 ¹ / ₂	7	5.1	8.3	7.6	15.3
			58	7	5.2	7.7	7.9	16.0
			62 ¹ / ₂	7	3.4	4.9	6.4	7.3
			66 ¹ / ₂	8	---	---	---	---
5	do.	21	24 ¹ / ₂	7	---	---	---	---
			28 ¹ / ₂	7	---	---	---	---
			32	7	---	---	---	---
			36 ¹ / ₂	7	---	---	---	---
			40 ¹ / ₂	8	---	---	---	---

¹ 1-plant accidentally killed.² Plants nearly dead⁴ All plants dead or dying.

EFFECT OF CONSTANT SOIL TEMPERATURE ON THE DEVELOPMENT OF BLACK SHANK

Four trials were conducted to determine the cardinal soil temperatures for the development of black shank caused by *Phytophthora parasitica* var. *nicotianae* Tucker (11). These temperatures may be defined as follows: Minimum, the lowest temperature at which symptoms of the disease appear; optimum, the temperature at which symptoms of the disease appear in the largest percentage of the plants within the shortest time after inoculation; maximum, the highest temperature at which symptoms of the disease appear.

Seven transplanted seedlings of the Round Tip variety, which is very susceptible to black shank, were inoculated and incubated at each temperature for each trial. The fungus used originated from a single-spore culture of *Phytophthora* isolated from tobacco at this station, and was grown on potato-dextrose agar or on steamed wheat. The fungus and the medium were mixed with the soil shortly before transplanting in two trials, and placed in contact with the stems below the surface of the soil in two others. The amount of inoculum was the same in all cans of each experiment, and was evidently sufficient in every trial to insure prompt infection at favorable tem-

peratures. One noninoculated check plant per compartment was left in each trial.

The plants used in the first three trials were placed in the cans in blocks of soil in which they had been transplanted a few days previously, so that the growth of the plants was not seriously interrupted. The plants used in the last trial were pulled and transplanted according to the usual field practice.

The time which elapsed between inoculation and the appearance of the symptoms of the disease was carefully observed. Diseased plants were recognized by permanent wilting, generally followed by the blackening of the stem above the soil and cork. Stunting of the inoculated plants, as compared with the check plant at the same temperature, was often noticeable before the wilting; the checks remained healthy in every instance.

The results of four trials with Round Tip plants are given in table 2. That temperature has an important effect on the pathogenic activity of the fungus is shown by the number of plants infected and the time required for the appearance of symptoms of the disease. The minimum temperature varied considerably with the age of the plants, from 16° C. or lower for newly transplanted seedlings to about 24° for plants inoculated 41 or 47 days after transplanting. The optimum was approximately 28°. The maximum was near 34°, but was not clearly defined, for a few plants died apparently from the combined effects of black shank and unfavorably high temperature.

TABLE 2.—*Effect of various constant soil temperatures on the development of black shank in tobacco plants of the Round Tip variety*

Trial No.	Soil temperature	Period of incubation before inoculation	Plants showing symptoms of black shank after indicated number of days						
			4	6	10	14	15	20	40
	°C.	Days	Number	Number	Number	Number	Number	Number	Number
1.....	12	47.....	0	0	0	0	0	0	0
	16		0	0	0	0	0	0	0
	20		0	0	0	0	0	0	0
	24		0	0	0	0	2	2	2
	27		2	4	6	6	6	6	6
	30		4	5	5	5	5	5	5
	34½		4	5	5	5	5	5	5
2.....	38½	41.....	0	0	0	0	0	0	0
	9½		0	0	0	0	0	0	0
	15		0	0	0	0	0	0	0
	20		0	0	0	0	1	1	1
	24½		0	0	0	0	6	6	6
	28½		6	6	6	6	6	6	6
	32½		4	4	4	4	4	4	4
3.....	37	0.....	0	0	0	0	0	0	0
	41½		(1)	(1)	(1)	(1)	(1)	(1)	(1)
	10½		0	0	0	0	0	0	0
	16		0	0	0	0	0	0	0
	20		0	0	0	0	0	0	0
	24½		0	0	0	0	0	0	0
	28		5	5	5	5	5	5	5
4.....	31½	0.....	0	0	0	0	0	0	0
	35		0	0	0	0	0	0	0
	38½		0	0	0	0	0	0	0
	16		0	0	0	3	3	3	3
	19		0	0	3	4	5	5	5
	22½		0	0	2	5	6	6	6
	25½		1	3	6	6	6	6	6
4.....	28	0.....	1	4	6	6	6	6	6
	31½		1	0	3	3	4	4	4
	34		1	1	1	1	1	1	1
	37		0	0	1	2	2	2	2
	37		0	0	1	2	2	2	2

¹ Plants nearly dead at time of inoculation.

The effect of soil temperature on the development of the disease is probably due to the effect of temperature on the rate of growth of the fungus either saprophytically in the soil or parasitically in the tissues of the host. In fact, the cardinal temperatures for the development of the disease correspond somewhat closely to those reported by Tisdale and Kelley (10) and Tucker (11) for the growth on culture media of the pathogen isolated by Tisdale at this station.

The number of plants available for observation in the experiments reported was limited by the capacity of the apparatus, but the general agreement of the results of the various trials indicates that the conclusions are fairly reliable. The plants inoculated several weeks after transplanting (table 2, trials 1 and 2) were grown at the same temperature at which they were held after inoculation. It is possible that the temperature at which the plants were grown had some influence on their susceptibility. This subject warrants further investigation.

RELATION OF SOIL TEMPERATURE TO THE DEVELOPMENT OF BLACK SHANK IN THE FIELD

The results of the experiments reported above, as well as those of Tisdale and Kelley (10), show that the temperature of the soil may affect the activity of the black shank organism in the field. Some further field observations may be of interest here.

In September 1932, Round Tip plants were transplanted in the trial plot at this station, where high percentage of the Round Tip plants in the check rows had died during the usual growing season which ended in July. These plants were still free from black shank when they were pulled for examination in November. This indicates that the pathogen had become inactive in the soil during the midsummer months. No soil-temperature records for these months are available. The following season, Round Tip plants transplanted in the same field in March remained healthy for several weeks. The first wilted plants were observed about the first of May, when the soil temperature at a depth of 2 to 4 inches ranged from about 20° C. at night to 24° during the day. This agrees closely with the minimum temperature (24°) reported above for the development of black shank in Round Tip plants inoculated several weeks after transplanting.

The problem of the survival, multiplication, and distribution of the black shank fungus under field conditions deserves further investigation.

SUMMARY AND CONCLUSIONS

Experiments were conducted to determine the cardinal soil temperatures for the growth of transplanted cigar-wrapper tobacco seedlings. The minimum and maximum were found to be approximately 9° and 40° C., respectively, and the optimal range from about 24½° to 32°.

Experiments were conducted to determine the effect of constant soil temperatures on the development of black shank (*Phytophthora parasitica* var. *nicotianae* Tucker) in Round Tip tobacco plants, which are very susceptible to the disease. The minimum temperature for infection was found to vary considerably with the age of the plants, ranging from 16° C. or lower for newly transplanted seedlings to about 24° for plants inoculated several weeks after transplanting. The optimum is about 28° and the maximum about 34°.

Observations on black shank in the field indicate that soil temperature is an important factor in the development of the disease.

LITERATURE CITED

- (1) CAMP, A. F., and WALKER, M. N.
1927. SOIL TEMPERATURE STUDIES WITH COTTON . . . Fla. Agr. Expt. Sta. Bull. 189, 32 pp., illus.
- (2) GODFREY, G. H.
1926. EFFECT OF TEMPERATURE AND MOISTURE ON NEMATODE ROOT KNOT. Jour. Agr. Research 33: 223-254, illus.
- (3) HAYES, H. K., EAST, E. M., and BEINHART, E. G.
1913. TOBACCO BREEDING IN CONNECTICUT. Conn. State Agr. Expt. Sta. Bull. 176, 68 pp., illus.
- (4) HILGARD, E. W.
1911. SOILS, THEIR FORMATION, PROPERTIES, COMPOSITION, AND RELATIONS TO CLIMATE AND PLANT GROWTH IN THE HUMID AND ARID REGIONS. 593, pp., illus. New York, London.
- (5) JOHNSON, J., and HARTMAN, R. E.
1919. INFLUENCE OF SOIL ENVIRONMENT ON THE ROOT-ROT OF TOBACCO. Jour. Agr. Research 17: 41-86, illus.
- (6) JONES, D. F.
1921. CONNECTICUT ROUND TIP TOBACCO: A NEW TYPE OF WRAPPER LEAF. Conn. State Agr. Expt. Sta. Bull. 228, pp. 285-292, illus
- (7) JONES, L. R., JOHNSON, J., and DICKSON, J. G.
1926. WISCONSIN STUDIES UPON THE RELATION OF SOIL TEMPERATURE TO PLANT DISEASE. Wis. Agr. Expt. Sta. Research Bull. 71, 144 pp., illus. ..
- (8) KINCAID, R. R.
1935. THE EFFECTS OF CERTAIN ENVIRONMENTAL FACTORS ON THE GERMINATION OF FLORIDA CIGAR-WRAPPER TOBACCO SEEDS. Fla. Agr. Expt. Sta. Bull. 277, 47 pp., illus.
- (9) TINDALE, W. B.
1922. TOBACCO DISEASES IN GADSDEN COUNTY IN 1922, WITH SUGGESTIONS FOR THEIR PREVENTION AND CONTROL. Fla. Agr. Expt. Sta. Bull. 166, pp. 72-118, illus.
- (10) ——— and KELLEY, J. G.
1926. A PHYTOPHTHORA DISEASE OF TOBACCO. Fla. Agr. Expt. Sta. Bull. 179, pp. 159-218, illus.
- (11) TUCKER, C. M.
1931. TAXONOMY OF THE GENUS PHYTOPHTHORA DE BARY. Mo. Agr. Expt. Sta. Research Bull. 153, 208 pp., illus.

THE DEVELOPMENT OF THE BARLEY SPIKE¹

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INTRODUCTION

A study of the morphological development of the barley spike has practical significance for the agronomist and should be of interest to the botanist. From a study of the morphological development of the barley spike the agronomist can determine the critical period at which spike development may be affected by the environment. Differences in the rate of differentiation and growth in early spike development give a suggestion of the reasons for variation in the mature spike. Finally, the manner of the adjustment of the spike to environmental changes is indicated. To the botanist the development of the barley spike furnishes an example of the sequence of differentiation in a grass stem.

Publications dealing with the development of the barley stem from germination to pollination are few. Two publications that deal with the development of the barley spike from the earliest stages to complete differentiation should be mentioned. Schuster² published a description and a set of drawings illustrating several steps in spike and spikelet development. More recently Noguchi³ has given a brief description of the spike and spikelet development and has illustrated some of the phases of development by drawings. He also gives data showing the length and breadth of the spike of 6-row and 2-row barley at different stages from 10 to 200 days old.

The illustrations accompanying the above-mentioned publications are line drawings and hence present more or less diagrammatically what is shown in this article by photographs. In addition, several stages of development are shown in this article that are not included by the above-mentioned authors.

MATERIALS AND METHODS

Both 2-row and 6-row barley were used in this study and the photomicrographs that best showed the successive steps in spike and spikelet development were chosen without regard to type. This was justified since both types follow the same sequence of development, the only exception being that the side spikelets of the 2-row barley do not develop as rapidly or as completely as the central spikelets.

The plants were grown in pots in the greenhouse, and when they were 20 days old daylight was supplemented with electric light from 500-watt bulbs placed about 3½ feet above the bench on alternate nights. These conditions of growth produced normal plants except that the electric light hastened the development of the spike.

¹ Received for publication Apr. 15, 1935; issued December 1935.

² SCHUSTER, J. ÜBER DIE MORPHOLOGIE DER GRASBLÜTE. *Flora* [Jena] 100: 213-266, illus. 1910.

³ NOGUCHI, Y. STUDIEN ÜBER DIE ENTWICKLUNG DER INFLORESCENZEN UND DER BLÜTEN BEI GETREIDE-PFLANZEN. *Jour. Col. Agr., Imp. Univ. Tokyo* 10: 247-303, illus. 1929.

At intervals of a few days, plants were pulled, taken to the laboratory, and the growing points dissected from the stem under the low power of a binocular microscope. Especially ground dissecting needles were used. The growing point was easily exposed and removed for photographing by carefully cutting and removing the leaves enclosing it.

Photomicrographs were taken with an upright camera, adjusted to fit over one side of the binocular microscope. It was necessary to construct a special light-tight collar to connect the microscope and camera. The eyepiece was left in the microscope when exposures were made.

Light for photographing was obtained from a microscope lamp fitted with a 200-watt bulb. A Florence flask filled with water containing sufficient copper sulphate to produce a light blue-green color was used as a condenser. This set-up gave a light of sufficient intensity to produce a good negative on commercial orthochromatic film with about 10 seconds' exposure.

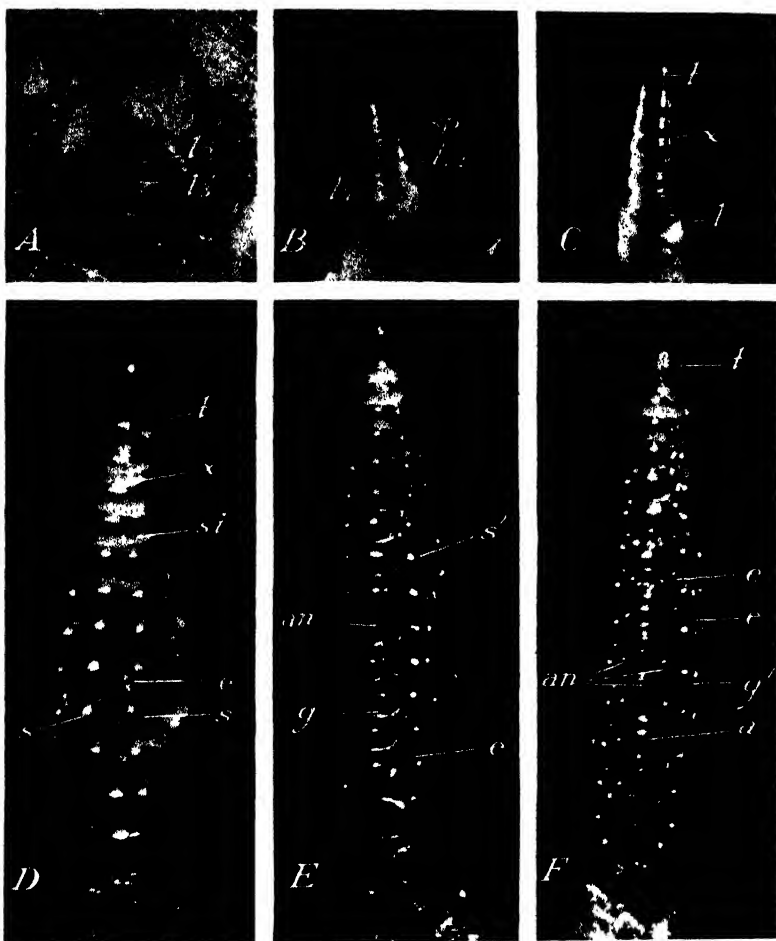
At first great difficulty was experienced in making photographs because of the very rapid drying of the growing points. Later, the growing points when dissected were placed on moist blotting paper in a small preparation dish and stored in a Petri dish lined with wet paper towels. Material handled in this way could be kept for 24 hours without any apparent deterioration. It was also found that specimens stored in the moist chamber for a period of time did not dry out as rapidly as those that had just been dissected.

Two methods of mounting the growing points for photographing were used. In one the specimen was mounted on moist blotting paper over black paper placed in the bottom of a preparation dish. The preparation dish was covered with a watch glass for the preliminary manipulations and focusing and then the watch glass was removed for the final focusing. The other method was to place a drop of petroleum jelly on a glass slide, mount the specimen in the jelly, and then place the slide over a black velvet background. Both methods of mounting were satisfactory except that in using the latter method more speed was necessary to complete the photographing before the specimen dried.

Growing points of barley are very difficult to photograph because they are colorless and nearly transparent. They were photographed against a black background with the light so placed that the high lights and shadows brought out the detail. In some cases in order to provide contrast, a stain composed of a mixture of 90-percent alcohol, a small amount of glycerin and basic fuchsin was applied to the specimen with a camel's-hair brush. The alcohol quickly evaporated, leaving the glycerin and stain in the folds of the various structures. Since the red stain photographed black the details of the structures were clearly outlined.

DESCRIPTION OF SPIKE DEVELOPMENT

In the resting stage of the barley grain the stem of the embryo is composed of only a few structures. These are the coleoptile and first leaf, which are the largest of the structures, the second and third leaf initials, the growing point, and a tiller bud in the axil of the coleoptile. The coleoptile and first leaf have been dissected from the embryo of the barley seed in plate 1, *A*, to show the second and third leaf



- A, A part of the embryo of a kernel of barley with the coleoptile and first leaf removed: *l*, Second leaf; *l*₂, third leaf. $\times 21$.
- B, Growing point of a barley stem in the 3-leaf stage: *l*₇, Seventh-leaf initial; *l*₁₂, twelfth-leaf initial; *sp.*, spike primordium. $\times 17$.
- C, Growing point of a 6-row barley stem in the 4-leaf stage, showing double ridges marking the beginning of spike differentiation: *l*, Leaf initial; *x*, double ridge; *t*, tip of spike. $\times 25$.
- D, Young spike of 6-row barley from a stem in the 5-leaf stage, showing the beginning of spikelet formation: *s*, Central spikelet; *s'*, side spikelet; *e*, empty glume; *si*, spikelet initial; *x*, lower of a double ridge; *t*, tip of spike. $\times 40$.
- E, Spike of a 6-row barley stem in the 5-leaf stage when the lemma and anthers begin to form. Stain has been used to make clear the position of the spike structures: *e*, Empty glume; *g*, lemma; *an*, anthers; *s'*, side spikelet. $\times 25$.
- F, A spike of 2-row barley from a stem in the 6-leaf stage showing a more advanced stage of glume, anther, and awn development. Stain has been used to mark out the structures: *a*, Awn; *an*, anthers; *g'*, lemma of a side spikelet; *s'*, and *e*, empty glume initials of the side and central spikelets respectively; *t*, tip of spike partially dried. $\times 25$.

initials. The growing point, which is hemispherical, is partly enclosed by the third leaf initial. By carefully removing the second and third leaves the primordium of the fourth leaf can be seen as a transverse ridge of the growing point.

By the time the second leaf is well grown nearly all of the leaves and leaf initials that the main stem will have can be found. The leaves range in size from those fully grown to leaf primordia just distinguishable as ridges at the base of the growing point. At this stage of stem development the growing point has just begun to elongate in preparation for spike differentiation. Up to this time the growing point has been short and hemispherical.

The growing point of a stem in the three-leaf stage is shown in plate 1, *B*. The leaves that enclosed this growing point ranged in size from the fully grown first and second leaves with the third leaf about 2 inches long down to the sixth leaf that was just large enough to enclose the growing point. The seventh-leaf initial is the basal one (pl. 1, *B*, l_7), while the last prominent ridge (pl. 1, *B*, l_{12}) is probably the last leaf that the stem would have produced. Above the twelfth-leaf initial is the part of the growing point from which the spike is differentiated.

The first indication of spike differentiation is the appearance of double ridges (pl. 1, *C*, x) instead of single ridges as was noted previously. At first the ridges are nearly equal in size, but the upper ridge of each pair grows more rapidly and from it the spikelets are formed. The lower ridge of the pair probably becomes the internode of the rachis, for apparently all of the spikelet structures arise from the upper of the pair of ridges.

Spikelet differentiation is first indicated by the appearance of two slight depressions in the transverse meristematic ridge. The very earliest stages of spikelet differentiation can be noted in the two spikelet initials at the base of the spike (pl. 1, *D*). Growth occurs in both sides and between the two furrows in preparation for the differentiation of the spikelet parts. Soon two little papillae, or empty glume initials, appear on opposite sides of each spikelet (pl. 1, *D*, e), but they appear first on the central spikelets.

Several stages of spikelet development are shown on the same spike (pl. 1, *D*). In the center portion of the spike the spikelet initials are quite prominent. The two transverse ridges at the base of the spike and some of the ridges at the top of the spike show only the first evidences of spikelet differentiation. The very tip of the spike is smooth and shows little evidence of the formation of ridges, while in plate 1, *D*, x , the lower of a pair of ridges can be seen.

The lemma is the first structure of the spikelet to differentiate (pl. 1, *E*, g). It appears as a distinct ridge across the spikelet initial and forms first upon the central spikelets in the central portion of the spike. Differentiation of the palea occurs somewhat later than that of the lemma, but since it is hidden by the other spikelet parts its development cannot be followed in gross dissections.

Soon three little papillae appear upon the meristem above the lemma (pl. 1, *E*, an , and *F*, an). These little papillae are the primordia of the anthers. The pistil is formed from that portion of the meristem located between the anthers, but it does not differentiate till considerably later than the anthers. The ovary differentiates

first, followed by the styles and last, the stigma, but no features of the differentiation of the pistil can be seen in gross dissections.

A side view of a young spike of barley is presented in plate 2, *A*, for the purpose of showing an early stage in the development of the rachis. In the early stages the internodes of the rachis are very short, but as the spike matures the internodes elongate. The degree of elongation determines spike density, a character used in the classification of barley varieties.

The awn begins its development as an outgrowth from the lemma (pl. 1, *F*, *a*). The awns and anthers grow quite rapidly and together with the empty glumes are soon the most conspicuous of the spikelet structures (pl. 2, *B* and *C*). The awn grows much more rapidly than the lemma and palea, which remain short with the anthers extending above them. About the time the last internode of the stem begins to elongate the glumes begin their growth and finally enclose the anthers and pistil. It should also be mentioned that in barbed varieties of barley the barbs can be seen on the awn at an early stage.

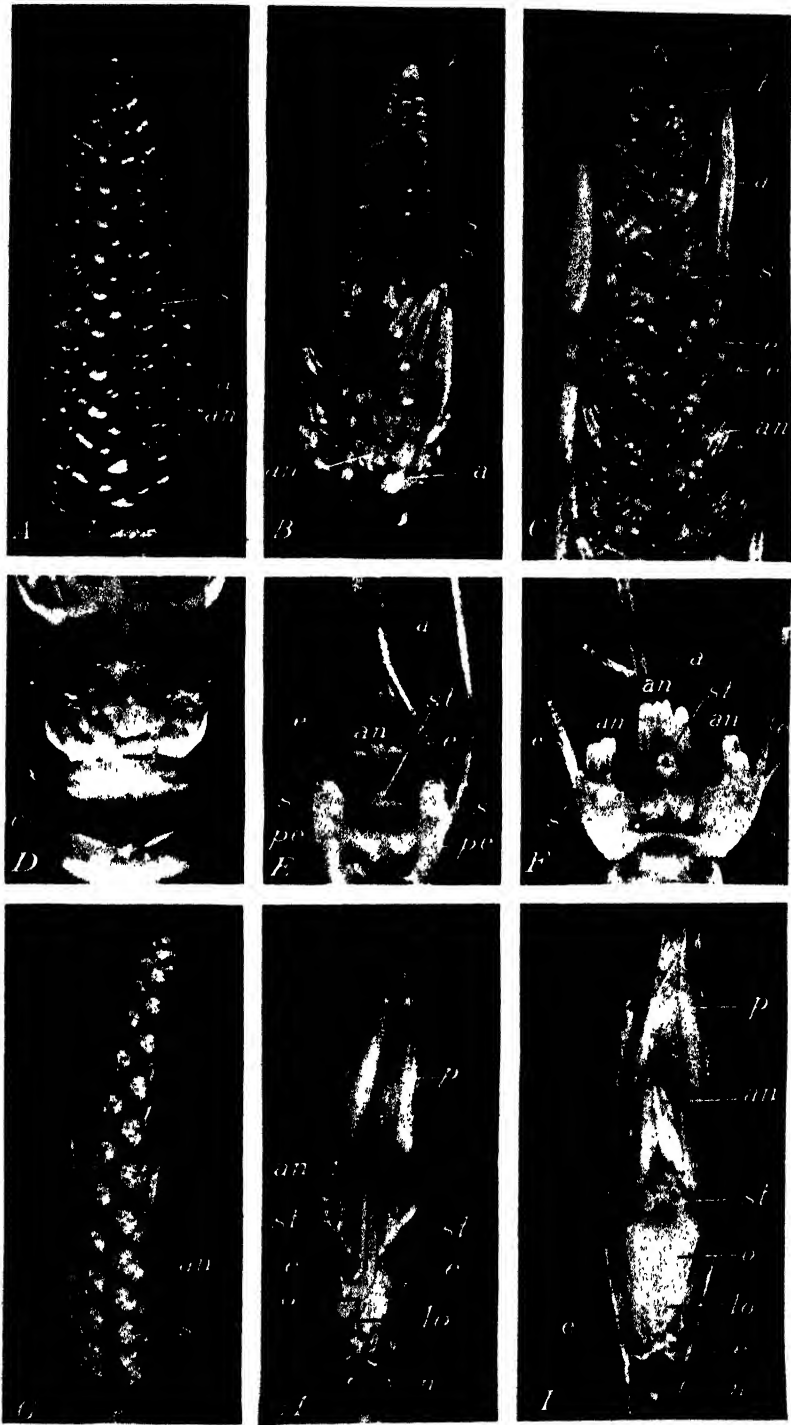
While the spike of barley shown in plate 2, *B*, was chosen principally to show the early development of the awn, it also shows the differentiation of a 6-row barley at the 6-leaf stage. A difference in development at the base and tip of the spike should be noted as well as the relative stages of differentiation in the side and central spikelets.

The relative size and degree of development of the tip of the spike and the spikelets a little lower on the spike should be noted (pl. 2, *C*, *t*). The very tip of the spike is at this stage a bit of undifferentiated meristem, and the spikelets at the uppermost 7 or 8 nodes of the rachis, as contrasted with the spikelets below, are much retarded in development. As has been pointed out, in the early stages of development the tip of the spike remains undifferentiated and smooth in outline. As the spike approaches maturity spikelet differentiation proceeds apically, but the last-formed spikelets never complete development, always remaining infertile and rudimentary. Rudimentary spikelets at the tip of the spike can be seen on any mature barley spike.

At the base of the spike is a structure called the collar (pl. 2, *D*, *c*). This structure is a distinct ridge of tissue circling the stem at the first node of the spike. A similar but less prominent ridge of tissue is found at the first node above the collar (pl. 2, *D*, *x*). So far as has been determined in these studies, the collar and the ridge at the node above are formed by two leaf initials that are just beginning to differentiate but are not far enough along to continue their develop-

EXPLANATORY LEGEND FOR PLATE 2

- A*, Spike of a 2-row barley stem in the 6-leaf stage, showing the rachis and a side view of anthers, awns, and side spikelets. Stain has been used to mark out structures: *an*, Anthers; *a*, awn; *s'*, side spikelet. $\times 30$.
B, A 6-row barley spike from a stem in the 6-leaf stage, illustrating awn development and the comparative development of side and central spikelets: *a*, Awn; *an*, anthers; *s*, central spikelet; *s'*, side spikelet; *t*, tip of spike. $\times 25$.
C, Part of a barley spike from the stem of a 2-row barley in the 5-leaf stage, showing the differentiation of the tip of the spike: *an*, Anthers; *e*, empty glume; *e'*, empty glume of side spikelet; *s'*, side spikelet; *a*, awn; *t*, tip of spike. $\times 25$.
D, Part of a spike from a stem in the 6-leaf stage, showing the collar at the base of the spike: *c*, Collar; *x*, second node of the rachis. $\times 30$.
E, Spikelet of a 2-row barley: *pc*, Pedicel; *s'*, side spikelet; *st*, style; *e*, empty glumes; *an*, anthers; *a*, awn. $\times 15$.
F, Spikelet of a 6-row barley: *s'*, Side spikelet; *e*, empty glume; *st*, style; *an*, anthers; *a*, awn. $\times 15$.
G, Spike of a 2-row barley: *s'*, Side spikelet; *an*, anther. $\times 8$.
H, Spikelet before pollination: *r*, Rachis; *lo*, lodicules; *o*, ovary; *e*, empty glumes; *st*, stigma; *an'*, anther; *p*, palea. $\times 5$.
I, Spikelet after pollination: legend as for *H*.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

ment as leaves at the time spikelet differentiation begins. They are arrested in their development and form the structures mentioned. While spikelets develop at the collar, they are late and usually rudimentary and sterile.

A spike of a 2-row barley well advanced in its development is shown in plate 2, *G*. The awns on the central spikelets are well developed but not fully grown and the anthers protrude well beyond the glumes. The side spikelets are small, without awns on the lemma, and rudimentary anthers extend slightly beyond the glumes. Much in evidence are the empty glumes upon which the barbs can be seen. Barbs can also be seen on the awns.

A spikelet of a 2-row barley (pl. 2, *E*) and a spikelet of a 6-row barley (pl. 2, *F*) are shown for comparison. Both spikelets were taken from stems in about the same stage of development. The last internode of the stem, the one to which the spike is attached, had just begun to elongate. The anthers and stigmas in the spikelets of the 6-row and in the central spikelet of the 2-row barley extend above the flowering glumes. However, just before the head emerges from the boot, the lemma and palea which up to this time have grown slowly begin to grow rapidly and soon enclose the anthers and stigmas. The anthers in the side spikelets of the 2-row barley have been enclosed by the flowering glumes. The side spikelets of the 2-row barley are much smaller than the central spikelet, are without awns on the lemmas, and are pedicellate. The side spikelets of the 6-row barley are nearly as large as the central spikelet, have awns on the lemmas, and are sessile. The side spikelets of the 6-row barley are fertile, while those of the 2-row barley are sterile. The stigmas in neither have branched (pl. 2, *E*, *F*, *st*).

Mature flowers before pollination and after pollination are shown in plate 2, *H*, and plate 2, *I*, respectively. The lemmas have been cut away to show the flower parts. Only one anther can be seen (pl. 2, *H*). The other two anthers are hidden in the folds of the palea. Before pollination the anther is not dehiscent, the stigmas are erect, branched, and feathery, the lodicules swollen and turgid, and the ovary small. After pollination the anthers are dehiscent, the stigmas are collapsed, the lodicules are shrunken, and the ovary has increased in size. The fertilized ovary after a period of growth and differentiation becomes the barley kernel.

Aside from the pistil and palea mentioned previously, two other spikelet structures, the rachilla and the lodicules, have not been followed in their differentiation and development in this study. They are so located that their differentiation and development cannot be shown.

DISCUSSION

That period in the morphological development of a head-bearing barley stem extending from germination to pollination can be divided into two phases. These phases can be determined approximately by examining the stem and more accurately by examining the growing point. In the first phase of development, the internodes of the stem do not elongate, leaves grow, leaf initials are the only structures differentiated from the growing point, and the growing point above the base remains smooth in outline but increases in length. The changes in the stem and the growing point which mark the transition

from the first to the second stage are shown by the beginning of internode elongation in the stem and the appearance of double ridges on the growing point. In the second phase of development, the internodes of the stem elongate, the spikelets and spikelet structures differentiate, increase in size, and complete their development in preparation for pollination.

It is interesting to note how the phases of stem development of a barley plant parallel each other not only in those stems that produce heads but in all stems even down to the tiller buds. When stems producing heads pass into the second phase, i. e., jointing and spike differentiation, it is not long before all stems on the plant follow in rapid succession. An examination of a plant in head shows even the growing point of the tiller buds to be in the process of spikelet differentiation.

Very early differences in the time of differentiation and rate of spikelet development are maintained and are reflected in the mature spike. Referring to plate 1, *D*, *E*, and *F*, the early differences will be noted. The spikelets in the middle of the spike are in advance of the basal spikelets and the basal spikelets are in advance of the tip, which is the last portion of the spike to differentiate. The central spikelets are more advanced in development than the side spikelets in both the 2-row and 6-row types. All of these differences are reflected in the mature spike. The best developed and heaviest kernels are in the middle portion of the spike, the basal kernels next, and the tip kernels are the lightest of all. The kernels in the central spikelets of the 6-row barley are heavier than those in the side spikelets. While the spikelets progressively develop at the tip of the spike, the spikelets remain rudimentary and do not bear kernels. Thus those spikelets that have an initial advantage in differentiation maintain this advantage throughout spike development.

Since the number of spikelets at the joints of the rachis is fixed, response to the environment during early differentiation takes place principally at the tip of the spike. The barley spike is an indeterminate inflorescence and does not terminate in a single spikelet as in wheat. Within limits a certain amount of response to growth conditions is made at the tip of the barley spike in the number of fertile spikelets. Some response can be made at the base of the spike, but the capacity for responding at this point is much more limited.

Although the lemma differentiates before the awn, the awn grows more rapidly. It is not until well along in the development of the spikelet that the lemma and palea become long enough to enclose the anthers and other flower parts. A possible explanation for this behavior is suggested by Kennedy⁴, who states that in the spikelet the awn corresponds to the leaf blade and that part of the glume below the insertion of the awn may be regarded as corresponding to the sheath of the leaf. He also states that of the three parts of the leaf the sheath develops last by intercalary growth which pushes up the blade. If the leaf parts and the spikelet parts are homologous as stated, then the slow growth of the lemma is in accord with development of the leaf sheath.

Up to the time that the anthers begin to differentiate, so far as the

⁴ KENNEDY, P. B. THE STRUCTURES OF THE CARYOPSIS OF GRASSES WITH REFERENCE TO THEIR MORPHOLOGY AND CLASSIFICATION. U. S. Dept. Agr., Div. Agronomy Bul. 19, 44 pp., illus. 1899.

varieties used were concerned, no difference could be noted between a spike of a 2-row and a spike of a 6-row barley. As development continues the discrepancy in the development of the central and side spikelets of the 2-row barley becomes more apparent. The side spikelets develop very slowly, remain rudimentary without awns and infertile. On the other hand, while the side spikelets of the 6-row barley are always slower in development than the central spikelets, they finally attain nearly the same size, have awns, and are fertile.

SUMMARY

A study was made of the morphological development of the spike of a 2-row and a 6-row barley by dissecting the growing points from the stems. Photomicrographs of the various stages are shown.

Stem development from germination to pollination can be divided into two phases in each of which the growth response of the stem and growing point are different. In the first phase the internodes of the stem remain short, the growing point produces only leaf initials, and the undifferentiated portion of the growing point elongates. The beginning of the second phase is marked by the elongation of the internodes of the stem and the appearance of double ridges on the growing point. In the second phase the internodes of the stem elongate and the spike and its parts differentiate and develop.

The order of differentiation of the various parts of the spike as far as could be seen in this study are: Spikelet initials, empty glumes, lemma, palea, anthers, awn, and pistil.

Early differences in the time and rate of differentiation of the spikelets in the different parts of the spike are maintained and account for some of the variation in size among the spikelets of the mature spike.

The barley spike is an indeterminate inflorescence, and with the number of spikelets at each joint of the rachis limited, some response to the environment can be made in the number of fertile spikelets at the tip of the spike.

THE SEQUENCE OF APPEARANCE, MOLT, AND REPLACEMENT OF THE JUVENILE REMIGES OF SOME DOMESTIC BIRDS¹

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PREVIOUS STUDIES

Interest in the problem discussed in this paper was first aroused in the authors by the occurrence of a heritable variation influencing the time of development of some of the remiges (flight feathers) in a flock of White Leghorns. A comparison of the sequence of appearance of the remiges in the chick of a normal and mutant stock (known as "retarded") led to findings regarding the order of appearance and the number of molts of these flight feathers which were not in agreement with previous records found in the literature. While this work was in progress Dunn and Landauer (1)² gave a detailed account of the sequence of replacement of the juvenile remiges in the Silver Spangled Hamburg fowl which the writers were able to confirm in studies on White Leghorns. A brief abstract of the data on the sequence of molt of the remiges in White Leghorns has been published (8).

The observations were later extended to other domestic birds to determine whether the regular manner of molt with respect to the members of the *Gallus* group but irregular with respect to the position of the feathers was found in other genera.

The work of Dwight (2) and Heinroth (3) indicate that some variability occurs in the sequence of molt of the secondary flight feathers of different species of passerine birds. Stone (7) also found a considerable lack of agreement among smaller land birds as to the order of molt of the flight feathers. The description by Rice, Nixon, and Rogers (6) of the sequence and number of molts of the remiges in White Leghorns does not agree with the writers' findings. It should be kept in mind that most of the observations cited on molt in wild birds were made on adults, whereas the present study is concerned primarily with the juvenile condition. The writers' studies indicate that the sequence of molt in gallinaceous birds differs somewhat in the juvenile condition from that reported by Marble (4) for the adult of *Gallus domesticus*. Marble (5) found it impossible to forecast the age at sexual maturity in White Leghorn females by the development of the primary wing feathers at 8 weeks of age.

METHODS AND MATERIAL

All of the species used in this study were a part of the stock carried at the Kansas Agricultural Experiment Station. As is indicated in the tabular data, the intervals between observations on the sequence of appearance and dropping of the remiges were varied in the different

¹ Received for publication Apr. 15, 1935; issued December 1935. Contribution no. 87, Department of Poultry Husbandry, Kansas Agricultural Experiment Station.

² Reference is made by number (italic) to Literature Cited, p. 470.

forms. As experience was gained the interval could be lengthened with less danger of confusion between new and old feathers. The use of dyes on the tips of the remiges aided in the recognition of the various molts. This is especially desirable in the early adult stage when members of three different sets of remiges may be present at one time.

In identifying the various remiges they were numbered anteriorly in the primaries and posteriorly in the secondaries. The writers have followed the lead of the poultrymen and called the first feather posterior to the primaries the axial rather than the first secondary. Its small size and distinctive behavior in time of appearance and molt would seem to justify placing it in a separate category. Since the secondaries appear irregularly there may be some difficulty for one inexperienced in identifying the different remiges. However, a close examination of the skin in which the remiges occur will usually solve the problem since the irregularly appearing axial and secondary no. 1 may be seen while yet embedded in the skin. The determination of the position of these two feathers will usually make it possible to identify accurately the other feathers at an early stage.

NUMBER OF REMIGES

In the various species studied the number of primaries was more constant than that of the secondaries. The forms studied—Single Comb White Leghorn, Single Comb Rhode Island Red, and Light Brahma chickens (*Gallus domesticus*); Pearl guineas (*Numida meleagris*); and Bronze turkeys (*Meleagris gallopavo*)—all showed 10 for the normal number of primaries. An extra primary was occasionally found in the various breeds of chickens observed. The eleventh primary occurred more frequently in the Light Brahmas and Rhode Island Reds than in the White Leghorns.

There was considerable variability in the number of secondaries both among breeds and individuals of a breed. The more usual number in White Leghorns was 14, in Rhode Island Reds 15, in Light Brahmas 16, in guineas 14, and in turkeys 16. The inner few secondaries were so reduced in size that they were not sharply differentiated from the tertiaries. In most cases data were taken only on the first 12 secondaries.

SEQUENCE OF EMERGENCE AND MOLT OF REMIGES

SINGLE COMB WHITE LEGHORN

The most extensive observations were made on Single Comb White Leghorns. Figure 1 shows the outline of the wing of an adult White Leghorn female indicating the numbers by which the remiges were identified in this study. Primaries nos. 1 to 7 and secondaries nos. 2 to 8 were usually present at hatching. Although they had grown several millimeters in length at time of hatching these feathers remained in their enclosing sheaths for several days. Progressing anteriorly in the primaries and posteriorly in the secondaries the new feathers appeared regularly as the chick aged. The axial and secondary no. 1 appeared late and there was considerable variability among individuals as to the age at which these feathers grew out. Figure 2 presents in graphic form the record of the mean age at which

the various remiges appeared and molted. Since the molted feathers were replaced immediately no distinction was made between time of molt and replacement.

The primaries which were not present at time of hatching appeared in very regular order at 10- to 14-day intervals. The intervals between appearance of the successive inner secondaries (nos. 8 to 12) were less uniform but the sequence was regular. The secondary no. 1 did not appear until the chick was about 2 weeks of age or not until the approximate time of the appearance of the tenth secondary and eighth primary. The axial feather did not emerge from the skin until about 4 weeks of age. This was the approximate time at which the twelfth secondary and the ninth primary appeared. It will be noted from figure 2 that the axial appeared only slightly before the first primary (no. 1) was molted.

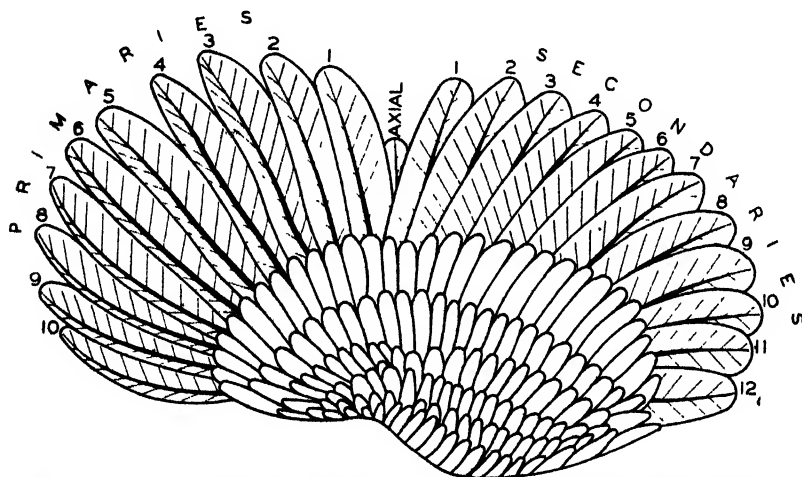


FIGURE 1.—Wing of a White Leghorn female showing system of numbering for the identification of the remiges. Only 12 secondaries are shown in this drawing.

THE FIRST MOLT

Beginning with feather no. 1, molt in the primaries progressed anteriorly in a very regular order. The secondaries were also molted in exactly the same order in which they appeared, beginning with no. 2 and being dropped in a regular manner from the outside in, or in the reverse order of the primaries. The secondary no. 1 and the axial were dropped at a much later date.

In practically all instances the corresponding flight feathers in the females appeared at a slightly earlier age than did those of the males. This was true of the molting of the remiges as well as of the time of their appearance. Since the number of secondaries was somewhat variable the observation on growth and molt included only feathers numbered 1 to 12. It is of interest to note from figure 2 that the time of emergence of the secondary no. 1 and the axial form a straight line with the series of the second set of primary feathers, the secondary no. 1 being at the top of the line and the primary no. 10 at the bottom. The same relationship was observed to hold between the

second secondary no. 1 and axial feather and the third set of primaries: It is not known whether this agreement of the behavior of these two feathers and the group of primaries has any significance.

SEQUENCE OF JUVENILE AND ADULT MOLT

Marble (4) has published a detailed account of the order of molt of the remiges in the adult fowl. In some respects there are close agreements of conditions found in the adult and the growing chick, while in other phases there is a rather wide divergence in sequence of molt. The first chick molt and the adult molt following a period of production agree in retention of the axial and secondary no. 1 until

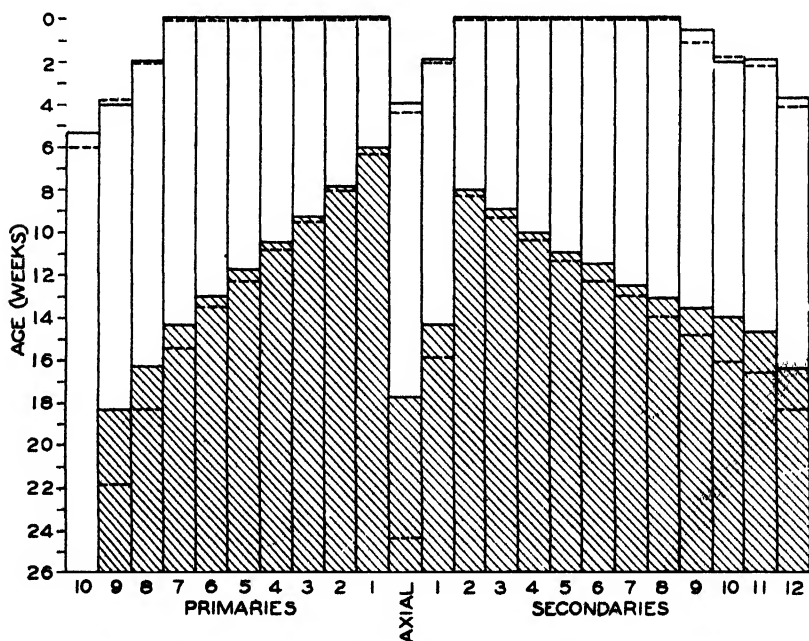


FIGURE 2.—Age at emergence and at molt of each remex of the White Leghorn. The upper outline of the graph indicates the age at which the remiges emerged. Those shown on the zero line were present at hatching. The lower gradations (diagonally lined) record the age at the time of molt of the remiges. The solid line is for females and the broken line for males. The space between the two solid or the two broken lines (of upper and lower graph) measures the period over which the feather was held before being molted.

the molt of the primaries and remaining secondaries is practically complete. There is a disagreement in that the inner small secondaries are the first to be molted in the adult while they are the last of the series of secondaries to be dropped by the chick. In both the chick and adult the usual procedure in the primaries is for them to be molted in regular order, beginning with the one (no. 1) adjoining the axial.

In a group of individuals of any one breed there was close agreement as to the age at which the various primaries and secondaries appeared and were dropped. This fact is well brought out in table 1. The data for the entire group of White Leghorns, presented in this table, show that the limits of time over which they varied in molting

any one flight feather seldom exceeded 2 weeks and that most of the feathers were dropped within the period of a week. The greatest variability occurred in the late-appearing axial, secondary no. 1, the smaller inner secondaries, and the tenth primary. The original tenth primary was frequently carried throughout much of the first adult year. Such birds can be recognized by the narrow, pointed, and much-curved appearance of this juvenile feather in the wing. Marble (5) reported that pullets carrying the original tenth primary over into the first laying year showed a tendency to mature at an early age.

TABLE 1.—Age distribution for the emergence and molt of remiges in 15 White Leghorn females

Age (weeks)	Birds having or molting primary no.—										Birds having or molt- ing axial	Birds having or molting secondary no.—													
	10	9	8	7	6	5	4	3	2	1		1	2	3	4	5	6	7	8	9	10	11	12	13	14
0.....	—	—	—	15	15	15	15	15	15	—	—	—	15	15	15	15	15	15	15	12	1	—	—	—	—
2.....	—	—	15	—	—	—	—	—	—	—	—	15	—	—	—	—	—	—	—	3	14	15	2	—	—
4.....	5	15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	12	4	—	—
6.....	10	—	—	—	—	—	—	—	—	1	15	—	—	—	—	—	—	—	—	—	—	1	8	—	—
8.....	—	—	—	—	—	—	5	14	—	—	—	—	14	8	—	—	—	—	—	—	—	—	2	—	1
10.....	—	—	—	—	2	12	10	—	—	—	—	—	1	7	14	8	4	—	—	—	—	—	1	6	—
12.....	—	—	—	7	13	3	—	—	—	—	—	—	1	—	—	11	12	7	4	2	—	—	—	7	—
14.....	—	—	12	8	—	—	—	—	—	—	—	11	3	—	—	—	3	8	10	11	10	—	—	1	—
16.....	—	12	3	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	1	2	5	11	6	4
18.....	1	14	3	—	—	—	—	—	—	—	—	8	—	—	—	—	—	—	—	1	—	—	4	6	8
20.....	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—
22.....	7	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
24.....	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

THE SECOND MOLT

In White Leghorns the usual procedure was for only one complete molt of the flight feathers to take place before sexual maturity was reached. In some cases the first molt was not complete at maturity because of the carry-over of the original outer primary. In practically all males examined and in some females there occurred a second molt. This molt was usually started at about 5 or 6 months of age. The sequence was identical with that of the earlier molt beginning at primary no. 1 and secondary no. 2, progressing outward in the primaries and inward in the secondaries. As in the preceding molt, the first primary to be dropped was released a little before the first secondary. In a group of 23 White Leghorn males studied, primary no. 1 was dropped in the second molt at the mean age of 23 weeks, secondary no. 2 at 27 weeks, primary no. 2 at 28 weeks, secondary no. 3 at 30 weeks, secondary no. 4 at 32 weeks, secondary no. 5 and primary no. 3 at 34 weeks, secondary no. 6 at 37 weeks, secondary nos. 7 and 8 at 39 weeks, and primary no. 4 at 40 weeks. Observations were discontinued at this age. Observations regarding the second molt were carried out on only a few females, but up to 36 weeks the molt had progressed as far as the fifth primary and the eighth secondary in some birds. The females were laying throughout this molt. In another group of 15 White Leghorns the second molt was started at 25 weeks of age, but the females showed no evidence of this molt at the termination of the observation when 34 weeks of age.

RHODE ISLAND REDS

As is seen from figure 3, the general sequence of emergence and molt of the remiges in Rhode Island Reds was very similar to that in White Leghorns. However, there were fewer flight feathers present at hatching than in White Leghorns and considerably more variability as to the age at which the missing feathers appeared. Comparisons of the time of emergence and molt of the remiges in the various breeds and species studied are shown in table 2. The records on the 20 Rhode Island Reds were taken at 1 day and at 2, 4, 6, 8, 12, 16, 20, and 24 weeks. In the slower feathering individuals

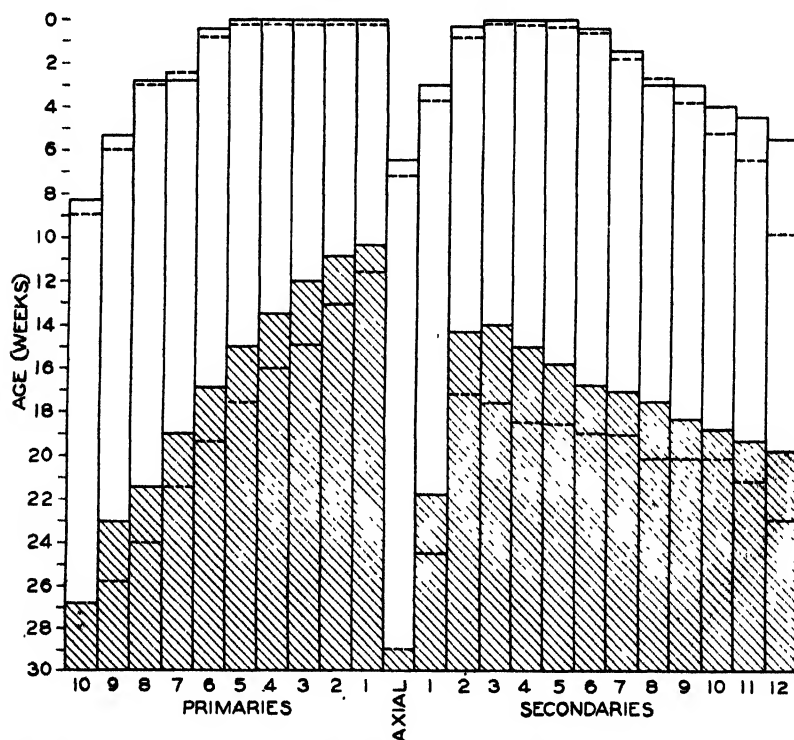


FIGURE 3.—Age at emergence and at molt of each remex of the Rhode Island Reds. The upper outline of the graph indicates the age at which the remiges emerged. Those shown on the zero line were present at hatching. The lower gradations (diagonally lined) record the age at the time of molt of the remiges. The solid line is for females and the broken line for males. The space between the two solid or the two broken lines (of upper and lower graph) measures the period over which the feather was held before being molted.

of the Rhode Island Reds and Light Brahmas the covert feathers appear before the remiges and may cause some confusion as to time of appearance of remiges. The first molt, although following the same order as in White Leghorns, was started and completed when the birds were about 6 weeks older. In no case had the axial molted at 6 months of age, and in several birds the original secondary no. 1 had not molted at this age, which was near sexual maturity. Both primary nos. 9 and 10 were the original feathers in most of the males at 6 months of age.

TABLE 2.—Comparison of several breeds and species of domestic birds in respect to the mean age at which the first remiges appeared

Feather no.	White Leghorn ¹		Rhode Island Red ²	
	Female	Male	Female	Male
Primaries:				
1	1.0 day	1.0 day	1.0 day	1.0 day
2	1.0 day	1.0 day	1.0 day	1.0 day
3	1.0 day	1.0 day	1.0 day	1.0 day
4	1.0 day	1.0 day	1.0 day	1.0 day
5	1.0 day	1.0 day	1.0 day	.2 weeks
6	1.0 day	1.0 day	.5 weeks	.8 weeks
7	1.0 day	1.0 day	2.8 weeks	2.5 weeks
8	2.0 weeks	2.0 weeks	2.8 weeks	3.0 weeks
9	4.0 weeks	3.8 weeks	5.3 weeks	6.0 weeks
10	5.3 weeks	6.0 weeks	8.3 weeks	8.9 weeks
Secondaries:				
Axial	4.0 weeks	4.3 weeks	6.5 weeks	7.2 weeks
1	2.0 weeks	2.0 weeks	3.0 weeks	3.7 weeks
2	1.0 day	1.0 day	.3 weeks	.8 weeks
3	1.0 day	1.0 day	1.0 day	1.0 day
4	1.0 day	1.0 day	1.0 day	.2 weeks
5	1.0 day	1.0 day	1.0 day	.3 weeks
6	1.0 day	1.0 day	.5 weeks	.5 weeks
7	1.0 day	1.0 day	1.5 weeks	1.8 weeks
8	1.0 day	1.0 day	3.0 weeks	2.7 weeks
9	.4 weeks	1.0 weeks	3.0 weeks	3.8 weeks
10	1.9 weeks	1.8 weeks	4.0 weeks	5.2 weeks
11	2.0 weeks	4.5 weeks	4.5 weeks	6.5 weeks
12	3.9 weeks	4.0 weeks	5.5 weeks	9.8 weeks

Feather no.	Light Brahma ³		Bronze turkey, ⁴ females and males ⁵	Pearl guinea ⁵	
	Female ⁶	Male ⁶		Female ⁶	Male ⁶
Primaries:					
1	(?)	(?)	(?)	(?)	(?)
2	(?)	(?)	(?)	(?)	(?)
3	(?)	(?)	(?)	(?)	(?)
4	(?)	(?)	(?)	(?)	(?)
5	(?)	(?)	(?)	(?)	(?)
6	(?)	(?)	(?)	(?)	(?)
7	2.6 weeks	3.8 weeks	(?)	(?)	(?)
8	4.0 weeks	5.1 weeks	(?)	3.6 weeks	3.7 weeks
9	5.2 weeks	6.8 weeks	5.9 weeks	4.0 weeks	4.2 weeks
10	8.0 weeks	10.9 weeks	7.7 weeks	6.0 weeks	6.0 weeks
Secondaries:					
Axial	6.2 weeks	6.0 weeks	5.0 weeks	4.0 weeks	4.0 weeks
1	4.6 weeks	4.7 weeks	4.0 weeks	(?)	(?)
2	2.4 weeks	2.9 weeks	(?)	(?)	(?)
3	(?)	2.9 weeks	(?)	(?)	(?)
4	(?)	2.7 weeks	(?)	(?)	(?)
5	(?)	(?)	(?)	(?)	(?)
6	(?)	(?)	(?)	(?)	(?)
7	(?)	(?)	(?)	(?)	(?)
8	2.4 weeks	3.1 weeks	(?)	(?)	(?)
9	2.8 weeks	3.8 weeks	(?)	(?)	(?)
10	4.0 weeks	4.0 weeks	(?)	1.5 weeks	1.5 weeks
11	4.0 weeks	4.0 weeks	(?)	2.0 weeks	3.1 weeks
12	4.4 weeks	5.6 weeks	(?)	4.0 weeks	4.2 weeks

¹ White Leghorns examined at 1 day of age and at 2-week intervals thereafter to 24 weeks.² Rhode Island Reds examined at 1 day of age and at 2-week intervals to 8 weeks and at 4-week intervals thereafter to 24 weeks.³ Light Brahmas examined at 2-week intervals to 8 weeks and at 4-week intervals thereafter to 24 weeks.⁴ Turkeys examined at 4, 6, 8, and 12 weeks of age.⁵ Guineas examined at 1, 4, 6, 8, and 12 weeks of age.⁶ The interrogation marks indicate that the feather was present at time of first examination; thus in the guinea females primaries nos. 1 to 5 were present when the first examination was made at 1 week of age, but it is not known exactly when they emerged.

LIGHT BRAHMAS

Twenty-one Light Brahmas were examined for the development of the remiges at 2, 4, 6, 8, 12, 16, 20, and 24 weeks of age. The females developed their various primaries at about the same age as

the Rhode Island Reds, but the Brahma males were slightly later in the development of these feathers than were the Reds. The secondaries developed very similarly in the Rhode Island Reds and Light Brahmas up to about the tenth feather. From the tenth secondary on the appearance of the successive feathers was much earlier in the Brahmas than in the Rhode Island Reds. This same tendency was observed in the first molt. The Light Brahma males showed little molt of the secondaries until the twentieth week at which time secondaries nos. 2 to 10 were found to be molted. In the Light Brahmas the tendency for several secondaries to be molted at one time was much more noticeable than in the other breeds of fowl examined. As in the case of the Rhode Island Reds, all Light Brahmas retained the original no. 10 primary at 24 weeks of age. This was also true of the axial in all males and most of the females.

GUINEAS

Twenty guineas were examined for development of the remiges at 1, 4, 6, 8, and 12 weeks of age. For number of remiges present at hatching and age of appearance of the additional ones, the guineas were similar to the White Leghorns (table 2). The first 7 primaries and 10 secondaries were present at the first examination made at 1 week of age. The observations were not made over so long a period as in the breeds of chickens, but the data were sufficient to indicate that the sequence and rate of molt was comparable to that in White Leghorns. In figure 4 are given the data for comparison of rate and sequence of molt in the various species studied. The failure of the axial and secondary no. 1 to appear and molt in order with the contiguous feathers was observed here as was the case in the three breeds of chickens studied. The remiges of the female guineas appeared and molted slightly ahead of those of the males, as was true of the breeds of chickens observed.

TURKEYS

Observations were made on 18 turkeys at 4, 6, 8, and 12 weeks. The data secured provided little information as to the time of appearance of many of the remiges, but a comparison of those present at 4 weeks of age showed that they must have appeared at about the same age as did those of the White Leghorns and guineas. Here again the axial and secondary no. 1 appeared much later than the adjoining secondaries, indicating that this irregularity is characteristic of many gallinaceous birds. A comparison of the age at which the remiges emerged is found in table 2. In some forms records were not taken at hatching and the interrogation marks indicate that the feather was present at the first examination. In figure 4 is a comparison of the time of first molt for the various remiges. In making the comparison note should be made of the differences in age at the last examination in the different species. The graphs for the turkeys and guineas represent only the upper segment of the ones for the different breeds of chickens.

MOLT OF EXTRA REMIGES

It is of interest to consider the time of appearance and molt of the supernumerary remiges in the birds studied. The number of secondaries was somewhat variable and it was difficult to say what were extra

feathers in this group. It is true, however, that beginning with the secondary no. 2, feathers of this group were dropped in a regular order from the outside in, so any supernumerary feathers were dropped following the regular series.

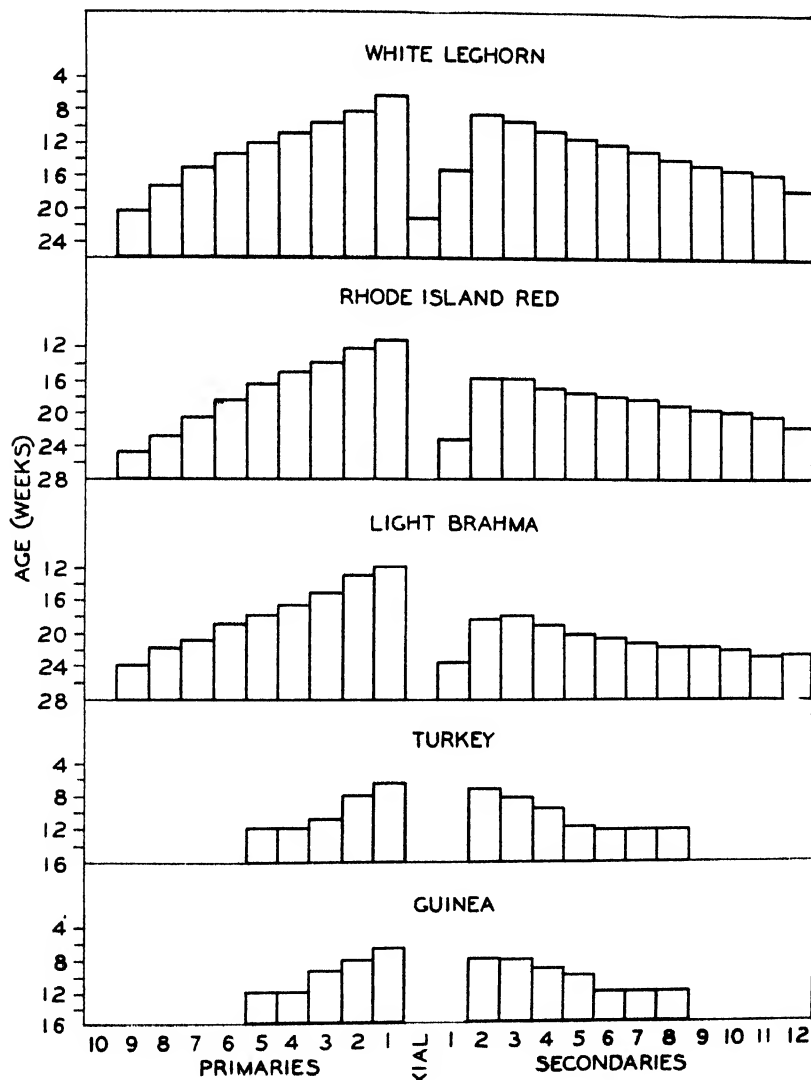


Figure 4.—Comparison of the age at, and sequence of, molt of the remiges in several species of domestic birds. Note that observations cover a much longer period in some species than in others, thus some graphs include only sections of the data given in others. Data are for mean of the two sexes.

In the case of the primaries the number seemed to be well established at 10. An occasional bird in all three breeds of chickens studied had 11. The eleventh feather always emerged after the tenth and was molted in the same order.

TIME OF MOLT AND RATE OF FEATHER GROWTH

There is little known as to the causal factors for molt in adults and the same is true of the juvenile molts. It is noted that although there are several primaries and secondaries present at time of hatching these feathers are not dropped at the same time. They are molted in a fixed and regular sequence. Those remiges appearing after hatching are molted in the same order in which they appear. This might indicate that the order of molt of the feathers present at hatching bears some relation to the sequence of their development in the embryo.

In order to determine whether a feather is molted after reaching a definite age, calculation was made of the period over which each of the original remiges was held. Since several feathers were present at hatching and since these feathers were molted in order with relation to position it would be expected that for those feathers present at hatching there would be a gradual increase in time held. Some idea of the relative periods over which the different remiges were held is gained by an examination of figure 2. The upper clear portion of each column indicates the length of period over which each original feather was held. The upper horizontal line marks the time of emergence and the lower one the time of molt. From table 3 it is seen that for primaries 1 to 7 and secondaries 2 to 8 the period increased by about 1 week for each successive feather in White Leghorns. Those appearing irregularly were found to vary considerably in period over which they were held, and the period was somewhat longer than for the other feathers. Those feathers which were molted regularly and which appeared after hatching were held for about 12 weeks in the females and slightly longer in the males.

The growth rate of the various remiges is of interest in view of the variation in age at which they appear and in relative length in the adult. Table 4 gives the length of each remex (first set) at 3 weeks after it appeared. The age at which the various remex lengths were recorded varied, depending on the age at which the feather appeared. The mean lengths are for the respective feathers in 11 female and 8 male White Leghorns. Measurements were taken at weekly intervals, but the data in table 4 are the means for the third (weekly) record. The number of feathers measured was not large enough to supply very conclusive results. Nevertheless, the data indicate that the rate of growth of the feathers bears no relationship to the time at which they emerge. There is some tendency toward an agreement between the position and rate of growth of the secondaries. Beginning with the axial feather, there is a somewhat regular decrease in rate of growth if we exclude the twelfth feather. An examination of figure 1 shows no correspondence between the adult length of the feather and its rate of growth. In considering the results in table 4, it should be kept in mind that secondary no. 1 does not appear until about the time that secondary no. 10 appears, and that the axial feather emerges along with the twelfth secondary. Secondaries 7 to 11 averaged somewhat shorter than the others after 3 weeks' growth. The axial and secondary no. 1 in both males and females showed considerably more rapid growth than did other remiges and these feathers were also considerably delayed in appearing.

TABLE 3.—*Period (weeks) between emergence and molt of the first set of remiges in White Leghorns*

Feather no.	Female		Male		Feather no.	Female		Male	
	Primaries	Secondaries	Primaries	Secondaries		Primaries	Secondaries	Primaries	Secondaries
Axial.....		13.7		20.0	7.....	14.4	12.4	15.5	13.0
1.....	6.0	12.3	6.3	13.8	8.....	14.3	13.1	16.3	14.0
2.....	7.9	8.1	8.0	8.3	9.....	14.3	13.2	18.0	13.8
3.....	9.3	8.9	9.5	9.3	10.....	16.1	12.1	19.3	14.2
4.....	10.4	10.1	10.8	10.3	11.....		12.7		14.5
5.....	11.7	10.9	12.3	11.3	12.....		12.5		14.3
6.....	13.1	11.5	13.5	12.3					

 TABLE 4.—*Comparison of lengths (millimeters) of remiges at the end of the first 3 weeks of growth in White Leghorns*

Sex and chick no.	Length of primary no. -										Length of axial	Length of secondary no.---											
	10	9	8	7	6	5	4	3	2	1		1	2	3	4	5	6	7	8	9	10	11	12
Female																							
923	69	73			78	85	87	87	83	78	66	92	78	72	74	74	73	70	62	63	67	71	73
924	66	75	63	64	68	72	74	73	70	60		87	76	65	64	63	60	61	60	57	58	55	60
927	69	81	63	69	75	75	75	78	75	68		99	60	68	68	67	65	66	61	56	58	49	58
928	78	89	66	75	82	85	83	82		78		86	95	74	73	72	73	71	67	54	58	58	68
929	78	88		75	81	83	83	83	78	76		90	93	73	73	73	70	70	68	63	58	58	54
930	66	80	68	71	75	76	77	77	76	68		88	89	68	68	67	67	63	60	53	63	51	52
935	75	84	71	76	84	85	85		82	75		78	94	76	75		73	71	70	65	60	64	54
937	70	80	66	68	76	73	77	77	73	68		80	82	66	66	65	64	64	60	56	50	55	60
943	80	70		74	77	80	80	80	80	70		86	78	70	70	68	69	68	64	63	60	64	66
944	81	68		74	80	82	81	80	77	67		75	81	73	72	71	71	69	65	64	58	61	71
945		74		62	70	70	70	71	68	55		72	77	63	62	60	57	58	55	50	45	47	48
Mean	73	78	66	71	78	79	80	78	76	68		85	82	70	70	68	66	67	64	59	56	58	60
Male																							
922	71	83	68		78	79	77	78	74	65		91	96	67	67	69	68	67	65	62	55	64	50
931	75	65	71	75	83	83	83	82	77	74		95	77	75	77	78	74	72	70	70	62	62	67
932	87	74		77	84	84	84	82	80	71		102	70	73	75	73	73	73	71	68	62	68	72
933	77	61	88	75	82	86	85	85	82	85		95	96	72	72	70		62	60	63	73	62	
934	70	59	86	71	76	81	82	82	80	72		85	87	70	70	68	67	58	62	57	70	57	
938	88	62	52	63	63	67	69	66	60			96	92	79	57	53		48	45	38	30		
941		72	78	63	68	71	73	69	68	66		72	62	62	63	62	58	56	48	38	48	72	
946			65	76	82	83	83	83	82	74			74	74	74	72	71	67	65	55	59		
Mean	76	72	74	70	77	79	79	79	76	70		94	85	72	69	69	64	62	59	56	60	62	69

SUMMARY

Three breeds of chickens (White Leghorn, Rhode Island Red, and Light Brahma), guineas, and turkeys all agree in having several primaries and secondaries present at hatching. In the White Leghorns, turkeys, and guineas more feathers were present at hatching and the missing feathers appeared earlier than in the Rhode Island Reds and Light Brahmas.

The irregular sequence of emergence and molt of the secondaries was found to be characteristic of all species of domestic birds examined.

One complete molt of the remiges usually took place during growth, but frequently the original primary no. 10 was carried unmolted by the sexually mature chicken.

A second molt, following the same sequence as the original one, was observed in some chickens when they approached sexual maturity.

There was considerable difference in period of time over which the various remiges were held before being molted.

LITERATURE CITED

- (1) DUNN, L. C., and LANDAUER, W.
1930. THE EXPRESSION OF THE SPANGLED PATTERN DURING GROWTH. Conn. (Storrs) Agr. Expt. Sta. Bull. 163: 31-46.
- (2) DWIGHT, J., Jr.
1900. THE SEQUENCE OF PLUMAGES AND MOULTS OF THE PASSERINE BIRDS OF NEW YORK. Ann. N. Y. Acad. Sci. (1900-1) 13: 73-360, illus.
- (3) HEINROTH, O.
1898. MAUSER UND VERFÄRBUNG DES FEDERKLEIDES DER VÖGEL. Sitzber. Gesell. Naturf. Freunde Berlin 1898: 9-16.
- (4) MARBLE, D. R.
1930. THE MOLTING FACTOR IN JUDGING FOWLS FOR EGG PRODUCTION. N. Y. (Cornell) Agr. Expt. Sta. Bull. 503, 42 pp., illus.
- (5) -----
1934. RELATION OF JUVENILE PLUMAGE TO GROWTH AND SEXUAL MATURITY. Poultry Sci. 13: 195-201.
- (6) RICE, J. E., NIXON, C., and ROGERS, C. A.
1908. THE MOLTING OF FOWLS. N. Y. (Cornell) Agr. Expt. Sta. Bull. 258, pp. [19]-68, illus.
- (7) STONE, W.
1897. THE MOLTING OF BIRDS WITH SPECIAL REFERENCE TO THE PLUMAGES OF THE SMALLER LAND BIRDS OF EASTERN NORTH AMERICA. Acad. Nat. Sci. Phila. Proc. 1896: 108-167, illus.
- (8) WARREN, D. C., and GORDON, C. D.
1931. THE GROWTH AND MOLT OF JUVENILE FLIGHT FEATHERS IN THE SINGLE COMB WHITE LEGHORN CHICKENS. (Abstract) Poultry Sci. 10: 404-405.

MACROSPOROGENESIS AND EMBRYOLOGY OF MEDICAGO¹

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INTRODUCTION

This study of the macrosporogenesis and embryology of alfalfa (*Medicago sativa* L.) is preliminary to an investigation of the causes of the failure of seed setting in certain strains of this species. Reeves (13)² gave a detailed account of microsporogenesis and traced the development of the ovule and of the embryo sac (14). He did not give the details of macrosporogenesis and did not continue the study through fertilization and the development of the embryo. Martin (11) described stages in the development of the embryo sac of alfalfa and compared these with corresponding stages in other Leguminosae. Ghimpu (7), Kawakami (10), Tschechow (17), Reeves (13), and Fryer (6) have reported the chromosome number of *M. sativa* as $n=16$, $2n=32$.

MATERIAL AND METHODS

The material for this investigation was collected from plants growing in the greenhouses of the Department of Genetics, Wisconsin Agricultural Experiment Station, during the late winters and early springs of 1932-34. These plants were producing an abundant crop of seed. For comparative purposes, material of seven other species of *Medicago*—*M. hemicycla*, *M. glutinosa*, *M. falcata*, *M. platycarpa*, *M. ruthenica*, *M. lupulina*, and *M. dzawakhetica*—was also collected. The first three have the same chromosome number as *M. sativa* and the remainder have half that number. Buds, young flowers, and fruits of various ages were placed in the following fixatives: La Cour's, Licent's, and Karpechenko's modification of Nava-shin's fluid. The best preparations were obtained from the material fixed in the two fixatives last named.

OBSERVATIONS

MACROSPOROGENESIS

Each ovule of *Medicago sativa* contains usually 2 or 3 primary sporogenous cells. These lie, in most instances, side by side in the nucellus (pl. 1, A), but occasionally they are end to end, appearing as a tetrad much as was described by Reeves (14). The primary sporogenous cells are easily identified by their large size and peculiar staining properties. They become more or less deeply embedded in the nucellus in consequence of some further division of the cells of the hypodermal layer. The presence of more than one sporogenous

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² Reference is made by number (italic) to Literature Cited, p. 476.

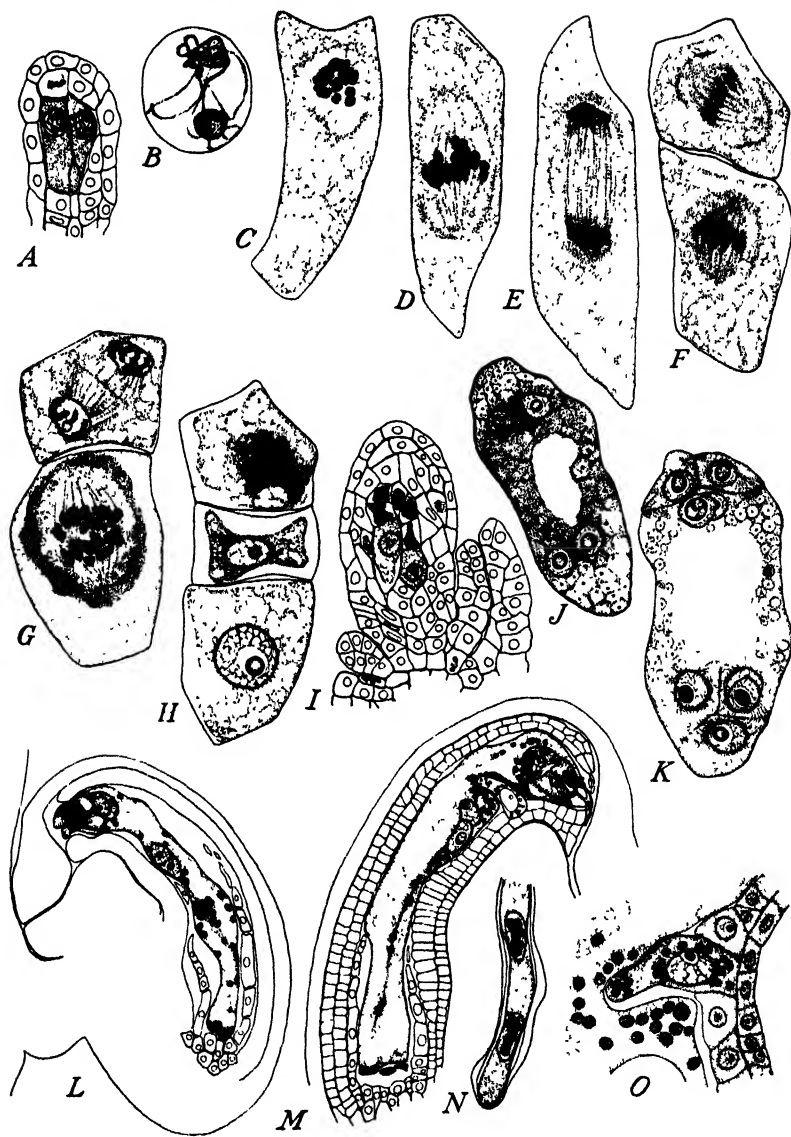
cell in *M. sativa* was noted by Martin (11) and by Reeves (14). The members of this genus vary in this respect. Guignard (9) found a single sporogenous cell in *M. arborea*. Corti (4) found a like condition in *M. hispida* and *M. arabica*, although occasionally more than one primary sporogenous cell was seen. There are usually two or more sporogenous cells in *M. dzawakhetica*, *M. falcata*, *M. glutinosa*, and *M. hemicycla*. One such cell is the rule in *M. lupulina*, *M. platycarpa*, and *M. ruthenica*, although in an occasional ovule two or more sporogenous cells are present.

The primary sporogenous cell functions as the macrospore mother cell without further division. It increases in size until it is about three times as long as broad. While the nucleus of the spore mother cell is passing through the early stages of the heterotypic division (pl. 1, *B*, *C*), the cytoplasm remains finely vacuolate. As meiosis progresses the cytoplasm at the ends of the cell becomes more vacuolate, and a dense zone surrounds the heterotypic and later the homeotypic spindles (pl. 1, *D-G*). The 16 pairs of chromosomes as seen at the multipolar spindle stage (pl. 1, *C*) and on both the heterotypic and homeotypic equatorial plates vary in size and shape. One pair is particularly prominent because of its large size. After the completion of the heterotypic division, a cell plate is formed and the macrospore mother cell is divided in such a manner that the chalazal daughter cell is about twice as large as the micropylar cell.

The axis of the homeotypic spindle in the chalazal cell is usually longitudinal, whereas the spindle in the micropylar cell may be transverse (pl. 1, *F*, *G*). In some instances they are formed simultaneously, in others the nuclear division in the chalazal cell either precedes or follows that in the sister cell (pl. 1, *G*, *H*). Occasional figures show an abortive equatorial plate in a micropylar cell which has already begun to disintegrate. Probably the occasionally observed row of 3 instead of 4 macrospores arises in this way. Most observers have found a linear row of 4 macrospores in the Leguminosae. In a few species, however, a row of 3 macrospores has been described. Guignard (8, 9) noted the formation of only 3 in *Phaseolus multiflorus* and in *Medicago arborea*. Weinstein (19) found the presence of 3 macrospores to be typical of *P. vulgaris*. This was brought about as the result of an abortive homeotypic division in the micropylar cell.

The spore mother cell, or in some instances more than one of the spore mother cells, of the apical ovule passes through these divisions first; corresponding divisions follow successively toward the base of the ovary. Microsporogenesis likewise is initiated in those stamens at the apex of the bud and gradually advances toward its base. Thus microspore tetrads may be found in stamens in the apical region of the bud, heterotypic spindles being present at the same time in the more basal stamens. In a few instances heterotypic spindles were found in apical ovules of buds in which the anthers at the apex contained microspore tetrads. Close examination revealed the presence of a tetrad of macrospores in the ovule as well as a spore mother cell bearing a heterotypic spindle, indicating that macrosporogenesis may lag in some of the sporogenous cells.

In numerous cases 2 or more tetrads are formed in a single ovule (pl. 1, *I*), and a few ovules were observed to contain 2 well-developed gametophytes. The chalazal macrospore of the row of 3 or 4 becomes



A, Portion of nucellus showing three primary sporogenous cells, $\times 330$; B, nucleus of macrospore mother cell, early spireme stage, $\times 1,365$; C, macrospore mother cell, heterotypic division, multipolar spindle stage, $\times 1,365$; D, same as C at early equatorial-plate stage, $\times 1,365$; E, same as C at telophase, $\times 1,365$; F, homeotypic equatorial-plate stage, $\times 1,365$; G, homeotypic division, anaphase stage in chalazal cell and cell plate formation in micropylar cell, $\times 1,365$; H, delayed mitosis in micropylar cell, large chalazal cell is functional macrospore, $\times 1,365$; I, ovule with two tetrads, one being in a stage similar to that of H, $\times 330$; J, 4-nucleate embryo sac showing remnants of spindles between nuclei and large central vacuole, $\times 1,365$; K, 8-nucleate embryo sac showing cell-plate formation, $\times 1,365$; L, ovule with maturing embryo sac, $\times 140$; M, embryo sac showing entrance of a pollen tube, an integumentary cell extending into the sac, $\times 146$; N, tip of pollen tube showing two male gamete cells, tube nucleus having disintegrated, $\times 1,365$; O, detailed drawing in integumentary cell extending into embryo sac M (note active nucleus and starch grains in this cell), $\times 683$. (Drawings made with Abbé camera lucida at table level.)



A, Egg apparatus and pollen tube shown in plate 1, *M*, $\times 725$; *B*, egg, showing male gamete nucleus closely appressed to egg nucleus, $\times 683$; *C*, stage in process of fusion of male gamete nucleus with egg nucleus, $\times 1,365$; *D*, zygote nucleus at spireme stage, $\times 1,365$; *E*, same as *D*, with two groups of chromosomes and two nucleoles present, $\times 1,365$; *F*, same as *D*, later stage (note position of satellite chromosomes), $\times 1,365$; *G*, chromosomes advancing to equatorial plate (note two groups), $\times 1,365$; *H*, oblique view of early zygotic spindle, chromosomes at left having advanced further on to the equatorial plate than those at the right, $\times 1,365$; *I*, lateral view of equatorial plate in zygote showing two groups of chromosomes, $\times 683$; *J*, zygotic equatorial plate, polar view, $\times 1,365$; *K*, same as *J*, lateral view, $\times 683$; *L*, nuclear division in zygote, telophase, $\times 683$; *M*, binucleate proembryo showing cell-plate formation with extra pollen tubes present, $\times 683$; *N*, 2-celled proembryo, $\times 683$; *O*, same as *N*, nucleus of apical cell dividing, $\times 683$; *P*, stage in formation of 4-celled proembryo, $\times 330$; *Q*, 4-celled proembryo, $\times 330$; *R*, 5-celled proembryo, $\times 330$; *S*, 6-celled proembryo, $\times 330$; *T*, 2-celled embryo with 5-celled suspensor, $\times 330$. (Drawings made with Abbé camera lucida at table level.)

the functional embryo-sac mother cell and the other spores disintegrate. This is in agreement with the reports of Martin (11) and Reeves (14). In *Medicago arborea* the lily type of embryo-sac development, wherein all 4 macrospore nuclei participate in the formation of the embryo sac, has been described by Guignard (9) and Herail, as quoted by Schnarf (15). An examination of the other species used for comparative purposes in the present study has shown that in each the chalazal spore alone develops into an embryo sac.

As a result of 3 nuclear divisions an 8-nucleate embryo sac is formed. The spindles of the second division apparently persist (pl. 1, *J*) so that after the last division the 4 nuclei at each end of the embryo sac are connected by spindle fibers (pl. 1, *K*). Cell plates are formed between the nuclei in such a manner that 3 of the nuclei at each end of the embryo sac are incorporated into a like number of cells, and the fourth nucleus remains in the central region. Thus a 7-celled embryo sac is formed consisting of an egg and 2 synergids at the micropylar end, 3 antipodal cells at the chalazal end, and, an elongated endosperm mother cell in the mid-region (pl. 1, *L*). The nucellus breaks down so that the micropylar end of the embryo sac comes to lie in direct contact with the inner integument. Mottier (12) observed cell-plate formation in the development of the embryo sac of *Lilium martagon*, and the writer (3) has described a similar phenomenon in *L. henryi*. Olive Rees (unpublished results) likewise observed cell-plate formation in the development of the embryo sac of *Solanum tuberosum*.

Small starch grains are to be observed in the four-nucleate embryo sac (pl. 1, *J*). They continue to increase in size and each grain has a definite shape at the time of the third nuclear division. Well-developed starch grains are abundant in the mature gametophyte.

The egg apparatus consists of three pear-shaped cells, the egg being somewhat larger than either synergid. A large vacuole appears in the basal region of each synergid, the small nucleus being embedded in the dense cytoplasm in the middle region of the cell. Just prior to fertilization the micropylar ends of the synergids elongate and extend into the micropyle. The cytoplasm of this region contains minute canals (filiform apparatus), which extend from the large basal vacuole toward the apex (pl. 2, *A*). The large egg nucleus is embedded in the dense cytoplasm in the basal region of the egg. This cell contains numerous starch grains, which are lacking in the synergids.

The antipodal cells, although usually well formed at the time of fertilization, disintegrate shortly thereafter. Reeves (14) has described early disintegration of the antipodal cells and finds that conductive cells, similar to the "Leitzellen" described by Ernst (5) for *Tulipa gesneriana*, extend before fertilization into the chalazal end of the embryo sac. No evidence of such structures has been found in the material used in this investigation.

The polar nuclei come to lie near each other in a region closely adjacent to the basal portion of the egg. They do not completely unite until the time of fertilization. Starch grains are abundant in the endosperm mother cell, especially in the dense cytoplasm near the fusion nuclei and near the egg apparatus. Comparatively few starch grains appear at the chalazal end of the endosperm mother cell and none in the antipodal cells (pl. 1, *M*).

FERTILIZATION AND DEVELOPMENT OF THE EMBRYO

Fertilization takes place between 24 and 27 hours after pollination. The pollen tube enters the embryo sac between the synergids and the egg. Usually only one pollen tube was seen, but in a few instances extra pollen tubes were present (pl. 2, *M*). The synergids are not broken down by the pollen tube as in *Phaseolus vulgaris* (Weinstein (18)), but persist for some time after fertilization.

The two male gametes are discharged from the pollen tube in the vicinity of the egg. As is shown in plate 1, *N*, each gamete nucleus is surrounded by a layer of cytoplasm which is distinct from that of the pollen tube. The tube nucleus may disintegrate early, but in a few instances it was found near the apex of the pollen tube (pl. 2, *A*). In the process of fertilization one male gamete nucleus becomes closely appressed to the egg nucleus, and the other unites with the fusing polar nuclei. The male gamete nuclei are not rounded as in *Phaseolus* (Brown (1)), but are more elongate as in *Melilotus* (Cooper (2)). Each contains a small nucleolus near one end.

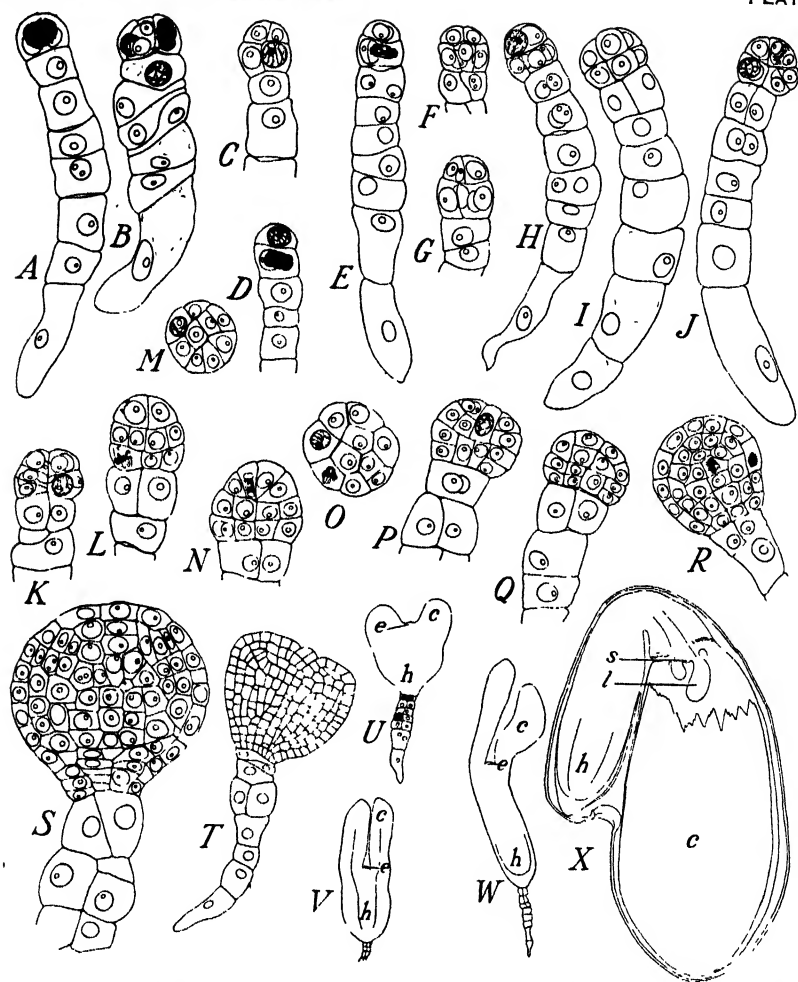
The small densely staining male gamete nucleus which is closely appressed to the less densely staining egg nucleus (pl. 2, *B*) now increases in size and its chromatic network tends to spread out so that ultimately the networks of the fusing nuclei are substantially similar (pl. 2, *C*) and the nuclear membranes disappear in the region of contact of the two nuclei. The nucleolus of the male gamete nucleus also enlarges to some extent but does not become as large as that of the egg nucleus.

Two distinct groups of chromosomes are now formed, one arising from the nuclear material of the male gamete and the other from that of the egg. These can be followed in plate 2, *D* to *G*. The two satellite chromosomes, one from each parent, are widely separated within the nucleus (pl. 2, *F*).

During the stages just described the fusion nucleus of the endosperm mother cell has divided once or sometimes twice, and by the time of the equatorial-plate stage in the division of the zygote nucleus commonly 2, and often 4, endosperm nuclei are present. The 2 chromosome groups of the zygote are usually somewhat separated on the equatorial plate (pl. 2, *G* to *I*), but occasionally the 2 groups cannot be distinguished (pl. 2, *J*, *K*).

The zygote divides transversely to form a two-celled proembryo consisting of an enlarged basal cell and a much smaller apical cell (pl. 2, *L* to *N*). Extra pollen tubes have been found in embryo sacs at this time. Plate 2, *M*, shows the two male gametes being discharged from a ruptured pollen tube.

The apical cell divides further so that a proembryo is formed consisting of 6 cells (pl. 2, *O-S*). Although both nuclei are preparing to divide in the 2-celled proembryo in plate 2, *N*, indicating that such a procedure perhaps may sometimes occur, usually only the apical cell divides (pl. 2, *P*). In one case, however, a mitotic figure was observed in the basal cell (pl. 2, *R*). According to Souèges (16), the apical cell of the 3-celled proembryo divides to form the true embryo in *Medicago lupulina*. Martin (11) found that it is the terminal cell of the 5-celled filament that forms the embryo in *M. sativa*. In all the material thus far examined a 6-celled proembryo is formed (pl. 2, *S*), and from the apical cell of such a proembryo the embryo is



A, Division forming 4-celled embryo, magnification here and unless otherwise noted, $\times 265$; *B*, cells of 4-celled embryo dividing transversely; *C-H*, stages of embryo development wherein either the first or second division was transverse, showing suspensor increasing in size; *I*, 8-celled embryo with attached 8-celled suspensor; *J*, *K*, cell divisions leading to 16-celled stage; *L*, two layers formed from basal tier of cells with apical cells undivided; *M*, transverse section of basal tier of embryo showing periclinal divisions; *N*, embryo showing nuclear division in apical tier; *O*, somewhat later stage than in *N*; *P*, *Q*, transverse division of cells of apical tier; *R*, *S*, older embryos becoming more spherical in shape; *T*, still older embryo, flattened apically and showing first evidences of primordia of cotyledons and epicotyl, $\times 257$; *U*, embryo with primordia of cotyledons (*c*), epicotyl (*e*), and hypocotyl (*h*), and suspensor at stage of greatest development, $\times 115$; *V*, embryo with elongating cotyledons and hypocotyl, $\times 60$; *W*, embryo bending in region of epicotyl, with suspensor disintegrating, $\times 60$; *X*, mature embryo with portion of upper cotyledon cut away to show primordia of leaf (*l*) and stem tip (*s*), $\times 30$. (Drawings made with Abbé camera lucida at table level.)

developed. The cells of the suspensor divide, and the suspensor increases in size.

Material from high seed-setting strains of alfalfa, collected at regular intervals after pollination shows considerable variation in embryo development. Stages in the division of the zygote and occasionally 2-celled proembryos are to be found 31 hours after pollination. The endosperm at this time contains from 2 to 4 nuclei. Two- to four-celled proembryos are present 48 hours after pollination, and 24 hours later these have so developed that they are composed of 5 or 6 cells. True embryos ranging from 2 to 16 cells in size are present 120 hours after pollination. Such variation in size of embryos is found not only between different ovaries, but also within a single ovary, where embryos were observed ranging from 4 to 12 cells in size.

Although there is an abundance of pollen tubes in the ovary at the time of fertilization, only about half or less of the ovules show the presence of proembryos at 31 and 48 hours after pollination. The cytoplasm of an embryo sac in which fertilization has not occurred remains apparently normal for a considerable time, the first evidences of disintegration being found in ovules collected 72 hours after pollination. This disintegration continues and ultimately the whole ovule becomes involved, so that at 120 hours after pollination the unfertilized ovules are small and very much shrunken. An examination of about a hundred ovaries revealed a range in number of ovules per ovary from 8 to 14 and a range in number of fertilized ovules per ovary from 1 to 6, the average being between 3 and 4.

The division of the apical cell in the formation of the embryo is usually parallel to the longitudinal axis of the proembryo (pl. 2, *T*). By two further divisions, the first vertical and the next transverse, an 8-celled embryo is formed (pl. 3, *A*, *B*, and *I*). Occasionally the first division is transverse and the second and third divisions are vertical (pl. 3, *C-H*). Periclinal divisions now occur which cut off the dermatogen (pl. 3, *I-L*). The cells of the embryo continue to multiply, forming first a spherical mass which later elongates and broadens at the apex (pl. 3, *M-T*). At this stage certain cell groups at opposite sides of the periphery of the apex as well as those in the basal region of the embryo become actively meristematic, and thus the cotyledons and hypocotyl are initiated (pl. 3, *T*, *U*).

The cotyledons appear as two outgrowths at the periphery of the apical region of the embryo. The epicotyl is a smaller outgrowth between them at the apex. The cotyledons elongate rapidly, becoming long, broad, and flattened (pl. 3, *V*). The hypocotyl also elongates, and during its further course of development the embryo curves in the region of the epicotyl (pl. 3, *W*) so that at maturity the cotyledons and hypocotyl are almost parallel (pl. 3, *X*). Provascular strands are differentiated early in the embryo (pl. 3, *V*). In the mature embryo, primordia of the first leaves are present near the apex of the stem. In the embryo represented in plate 3, *X*, a portion of one cotyledon is cut away to show the stem tip.

SUMMARY

Each ovule of *Medicago sativa* contains usually 2 or 3 primary sporogenous cells.

The primary sporogenous cells function directly as macrospore mother cells.

In consequence of the two meiotic divisions, the macrospore mother cell produces a row of four macrospores.

Occasionally only three cells are produced as a result of meiosis, the micropylar cell possessing an abortive homeotypic division spindle.

Usually 1, occasionally 2, and sometimes 3 macrospore tetrads are found in a single ovule.

The chalazal cell develops into an 8-nucleate, 7-celled embryo sac; the other macrospores disintegrate.

One embryo sac is usually formed in an ovule; occasionally two are present.

The apices of the synergids elongate into the micropylar canal, and a distinct filiform apparatus is present.

Fertilization takes place, under greenhouse conditions, between 24 and 27 hours after pollination.

The pollen tube enters the embryo sac between the synergids, neither of which disintegrates until later.

The antipodals persist for some time after fertilization.

In the course of gametic union, the chromosomes of the two gametes remain in two more or less distinct groups until the equatorial-plate stage of the first division of the zygote nucleus.

The zygote by transverse divisions forms a filament of six cells. The embryo develops from the terminal one of these cells. The other five cells by a few further divisions form the suspensor.

The embryo develops in a typical manner.

Although there is an abundance of pollen tubes present, less than half the ovules in an ovary show signs of fertilization. In heavy seed-setting lines of alfalfa there is an average of 3 to 4 seeds per pod, whereas 10 to 12 ovules are present in each ovary.

LITERATURE CITED

- (1) BROWN, M. M.
1917. THE DEVELOPMENT OF THE EMBRYO-SAC AND THE EMBRYO IN *PHASEOLUS VULGARIS*. Bull. Torrey Bot. Club 44: 535-544, illus.
- (2) COOPER, D. C.
1933. MACROSPOROGENESIS AND EMBRYOLOGY OF *MELILOTUS*. Bot. Gaz. 95: 143-155, illus.
- (3) ———
1934. DEVELOPMENT OF THE EMBRYO SAC OF *LILIUM HENRYI*. Natl. Acad. Sci. Proc. 20: 163-166, illus.
- (4) CORTI, R.
1930. PRIMI RISULTATI DI RICERCHE SULLA EMBRIOLOGIA E LA CARIOLOGIA DE ALCUNE LEGUMINOSE. Nuovo Giorn. Bot. Ital. (n. s.) 37: 278-279.
- (5) ERNST, A.
1901. BEITRÄGE ZUR KENNTNISS DER ENTWICKELUNG DES EMBRYOSACKES UND DES EMBRYO (POLYEMBRYONIE) VON *TULIPA GESNERIANA* L. Flora [Jena] 88: [37]-77, illus.
- (6) FRYER, J. R.
1930. CYTOLOGICAL STUDIES IN *MEDICAGO*, *MELILOTUS* AND *TRIGONELLA*. Canad. Jour. Research 3: 3-50, illus.

- (7) GHIMPU, V.
1928. CONTRIBUTION À L'ÉTUDE CARYOLOGIQUE DU GENRE *MEDICAGO*.
Compt. Rend. Acad. Sci. [Paris] 187: 245-247, illus.
- (8) GUIGNARD, L.
1881. SUR L'ORIGINE DU SAC EMBRYONNAIRE ET LE RÔLE DES ANTIPODES.
Bull. Soc. Bot. France 28: 197-201.
- (9) ———
1881. RECHERCHES D'EMBRYOGÉNIE VÉGÉTALE COMPARÉE. 1^{re} MÉMOIRE: LEGUMINEUSES. Ann. Sci. Nat., Bot. (6) 12: 5-166, illus.
- (10) KAWAKAMI, J.
1930. CHROMOSOME NUMBERS IN LEGUMINOSAE. Bot. Mag. [Tokyo] 44: 319-328, illus. [In Japanese and English.]
- (11) MARTIN, J. N.
1914. COMPARATIVE MORPHOLOGY OF SOME LEGUMINOSAE. Bot. Gaz. 58: 154-167, illus.
- (12) MOTTIER, D. M.
1898. UEBER DAS VERHALTEN DER KERNE BEI DER ENTWICKELUNG DES EMBRYOSACKS UND DIE VORGÄNGE BEI DER BEFRUCHTUNG. Jahrb. Wiss. Bot. 31: [125]-158, illus.
- (13) REEVES, R. G.
1930. NUCLEAR AND CYTOPLASMIC DIVISION IN MICROSPOROGENESIS OF ALFALFA. Amer. Jour. Bot. 17: 29-40, illus.
- (14) ———
1930. DEVELOPMENT OF THE OVULE AND EMBRYO SAC OF ALFALFA. Amer. Jour. Bot. 17: 239-246, illus.
- (15) SCHNARF, K.
1931. VERGLEICHENDE EMBRYOLOGIE DER ANGIOSPERMEN. 354 pp., illus. Berlin.
- (16) SOULEGES, R.
1929. RECHERCHES SUR L'EMBRYOGÉNIE DES LÉGUMINEUSES. Bull. Soc. Bot. France 76: 93-112, 338-346, 527-540, illus.
- (17) TSCHETCHOW, W. [CHEKOV, V.]
1930. KARYOLOGISCH - SYSTEMATISCHE UNTERSUCHUNG DES TRIBUS GALEGEAE, FAM. LEGUMINOSAE. (VORLÄUFIGE MITTEILUNG.) Planta 9: 673-680, illus.
- (18) WEINSTEIN, A. J.
1926. CYTOLOGICAL STUDIES ON *PHASEOLUS VULGARIS*. Amer. Jour. Bot. 13: 248-263, illus.

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INFLUENCE OF THE INGESTION OF COLOSTRUM ON THE PROTEINS OF THE BLOOD SERA OF YOUNG FOALS, KIDS, LAMBS, AND PIGS¹

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INTRODUCTION

In a project concerned with the artificial feeding of young farm animals from birth to the end of the suckling period, at the United States Animal Husbandry Experiment Farm at Beltsville, Md., a consideration of the importance of colostrum to the young of the different species became of great interest. There is considerable evidence in the literature, principally from the laboratories of Smith (11, 16, 17, 18, 19),³ indicating that colostrum, or its equivalent in the transfer of certain types of immunity, is essential for the rearing of healthy calves. It further appears, from the work of Orcutt and Howe (14), that in the calf at least the absorption of antibodies from the colostrum does not take place to any considerable extent except in conjunction with absorption of unchanged globulins from the colostrum ingested. The possibility that the direct absorption of globulins by the new-born animal may have some physiological significance aside from the transfer of any passive immunity should not be overlooked.

The author, therefore, has regarded the changes in the protein composition of the blood of the new-born animal following the ingestion of colostrum as a probable index of the absorption of colostrum and perhaps as some measure of the peculiar benefits available to the animal from the colostrum. This paper presents data in regard to the changes, which result from colostrum ingestion, in the protein concentration and in the distribution of protein fractions in the blood sera of young foals, kids, lambs, and pigs.

REVIEW OF LITERATURE

The function of colostrum in the early development of the young animal has been ascribed at times to its laxative properties and again to its high nutritive value, but more often to its role in the transfer of passive immunity from dam to suckling. Following the classic investigations of Ehrlich (4) on the transfer in mice of maternal immunity to certain phytotoxins, by means of milk, many studies

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³ Reference is made by number (italic) to Literature Cited, p. 459.

were made on the transfer of antibodies in milk. However, the interpretation of results from these studies was confused by the fact that in some of the species used there was immunization of the young in utero, whereas in others the young were born without the immune bodies which were present in the blood of the dam.

The literature on the many aspects of the subject of transfer of maternal immunity has been comprehensively reviewed by Braun, Hofmeier, and Holzhausen (2, *pp.* 1109-1146), and reference is made in the present paper only to such investigations as are thought to have an immediate bearing on the interpretation of the results obtained in the study.

In 1922 Smith and Little (16) published the results of the first of a series of investigations which definitely established the importance of colostrum for the survival of the new-born calf. They showed that from 75 to 80 percent of calves deprived of colostrum failed to survive, whereas the control animals, which had received colostrum, all lived. These authors concluded (16, *p.* 187)

that the function of the colostrum is essentially protective against miscellaneous bacteria which are harmless later when the protective functions of the calf have begun to operate and accumulate energy.

Howe (6) observed that the blood of the calf at birth contained neither euglobulin nor pseudoglobulin I in appreciable quantities, but that the ingestion of colostrum by the animal at any time within 2 days after birth resulted in the rapid appearance of these protein fractions in the blood. Orcutt and Howe (14) furnished evidence of the association of certain agglutinins with the globulin fractions in cow's colostrum and further demonstrated the simultaneous appearance of these globulin fractions and of the associated agglutinins in the blood of the new-born calf after the ingestion of the colostrum. These authors postulated a direct absorption of colostrum globulins together with the associated antibodies.

Lewis and Wells (10) repeated on the human infant the study which Howe made on the new-born calf. They found that in contrast to the calf, the infant has at birth a quantity of pseudoglobulin per cubic centimeter of blood comparable to that of the adult, and lacks only euglobulin. These findings were confirmed by Boyd (1), who further demonstrated that after the ingestion of colostrum there is a definite, although not great, increase in the quantity of serum euglobulin.

Kuttner and Ratner (9), in a study of the permeability of the human placenta to diphtheria antitoxin, found that the concentration of antitoxin in the blood of the umbilical cord of the infant corresponded to that in the blood of the mother. They observed no increase in antitoxin attributable to colostrum ingestion, and concluded that in the feeding of infants the significance of colostrum is not comparable to that in the feeding of calves as shown by the work of Smith and his associates (11, 14, 16). They considered the difference in the globulin content of the blood of infants and of calves at birth and the differences in transmission of immune substances in utero in the two species as probably arising from differences in the histological structure of the placentae of these species. They pointed out that there was apparently a greater degree of permeability for passage of antibodies and of serum globulins in species in which the placenta interposes only one layer of cells between the maternal and the fetal circulations, as in man, apes, and rodents, than in animals in which there are two or more cell

layers between the maternal and the fetal bloods. Later work on the transference of immunity in certain farm animals, reported in the following paragraphs, lends support to their conclusions that in species having histologically a more complex barrier between maternal and fetal circulations than the placenta chorialis, there is no placental transfer of immune substances.

Mason, Dalling, and Gordon (12), in a study of the transference of immunity to lamb dysentery in sheep, also carried out some experiments on the mare and the cow. They found no placental transmission of antitoxin from dam to young in the ewe, cow, or mare, but a passage of much antitoxin into the colostrum of these animals. These authors further demonstrated the subsequent appearance of the antitoxin in the blood of the new-born young after the ingestion of colostrum containing the antitoxin.

Nelson (13), similarly, has shown that new-born pigs born of sows immune to vaccinia virus have no immune substances to this virus until after the ingestion of colostrum from their dams.

ANIMALS USED AND THEIR MANAGEMENT

In this study, 10 foals, 3 kids, 6 lambs, and 4 pigs were used. Four of the foals were allowed to suckle their dams, 3 foals were fed measured quantities of colostrum from their dams, and 3 foals were fed a mixture of dried cow's milk, sugar, and water, which had a total protein concentration approximating that of fresh mare's milk. One of the kids was allowed to suckle its dam, one was fed measured quantities of colostrum from its dam, and the other was fed 4 ounces of sheep colostrum for the first 3 hours and only goat's milk after the third hour. Four of the lambs were allowed to suckle their dams, and two received a mixture of dried cow's milk in water. Of the 4 pigs, all litter mates, 2 were allowed to suckle their dam and 2 were placed with a sow which had farrowed 8 days previously.

All the young animals used in this study were delivered under observation, and unless a sample of blood was obtained from the umbilical cord when it was broken, each animal was immediately removed from its dam until the first sample of blood was drawn. If the animal was to be allowed to suckle, it was then placed with its dam or with a foster dam. If it was to be hand fed, it was rubbed dry and placed in a suitable stall or pen and fed from a bottle.

EXPERIMENTAL MATERIAL AND METHODS

BLOOD SAMPLES

Protein analyses were made of blood sera obtained from the new-born animals immediately after delivery and on blood sera obtained from the same animals at intervals after the ingestion of colostrum. Corresponding data were obtained from other new-born animals which received, in place of colostrum, either milk of the same species, colostrum of another species, or a mixture of dried cow's milk and water.

The first blood sample from each of foals 1 and 2 consisted of 15 or 20 cc of blood taken from the umbilical cord as it was severed just after delivery. All other blood samples from the foals, as well as all blood samples from the kids and lambs, consisted of 8 or 10 cc of blood drawn from a jugular vein with a sterile 16-gage needle.

Blood samples from the young pigs were drawn from the left ventricle of the heart by means of heart puncture. Not more than 6 cc of blood was drawn from a pig at any one time.

Because the sample of blood required for analysis was relatively large for so small an animal as the young pig, and because the method of drawing it was necessarily somewhat drastic for use on a new-born animal, it was planned to bleed the pigs only on alternate days. Of the two pigs allowed to suckle their dams, pig 1 was to be bled the first day, pig 13 the second, pig 1 the third, etc. Unfortunately, pig 1, which was bled on the first day, was killed by the sow on the third day before the second sample of blood was obtained from it. Of the 2 pigs placed with a sow which had farrowed 8 days previously, pig 2 was to have been bled the first day, pig 4 the second, pig 2 the third, etc., but both animals died on the third day. However, the assumption that the serum of pig 13 had at birth a protein composition similar to that of its three litter mates seems warranted; therefore, pig 13 has been used as representative of the effect of colostrum ingestion.

No anticoagulant was used in the collection of the blood samples; hence, all determinations were made on serum. For the determination of the protein fractions in the blood serum, Howe's micromethod (5) was used. In view of his later work (8) on the precipitating capacity of certain salts for the globulins of blood serum, 1.00, 1.25, and 1.50 volume molar concentrations of sodium sulphate were used instead of the 14.2, 18.4, and 21.5 percent concentrations formerly employed by him for the fractionation of the globulins. Eight percent of trichloroacetic acid was used for the precipitation of total proteins. All nitrogen determinations were made by the technique of Pregl (15). The modified Parnas-Wagner micro-Kjeldahl apparatus described by Clark and Collip (3) was used.

COLOSTRUM

As a means of determining whether there is any correlation between the protein concentration of the colostrum ingested and the amount of increase in serum proteins of the young animal, protein analyses were made in most cases on the colostrum which was ingested by each animal, although for only 3 foals and 2 kids is there a record of the quantity of colostrum ingested.

Samples of colostrum from the dams suckled by young under observation consisted of from 30 to 50 cc of colostrum drawn at one milking. In every instance, the first sample of colostrum from an animal was obtained before the new-born young was permitted to suckle. The samples taken for analyses from the colostrum which was later fed by hand to the experimental animals represented the product of several milkings.

All samples of colostrum were centrifugalized to remove fat and colostic bodies, and the fat-free or skimmed colostrum, which was pipetted from under the cream, was used for the protein determinations.

Protein precipitations were made according to Howe's method for the determination of the proteins in colostrum (7) with the modification later suggested by him that volume molar concentrations of sodium sulphate be substituted for the volume percent concentrations originally used. This method for the determination of the proteins of

colostrum was evolved for use with cow's colostrum. However, in fractioning the proteins of the milk of the mare, the ewe, and the doe (goat) in the present study, difficulty was encountered in separating casein, pseudoglobulin I, and pseudoglobulin II by this method. For this reason pseudoglobulin I and pseudoglobulin II are recorded together as one fraction. This fraction was calculated as the difference in the nitrogen content of the filtrates after precipitation with 1.50 molar sulphate and after precipitation with 1.00 molar sodium sulphate acidified with acetic acid.

DATA AND DISCUSSION

FOALS

Table 1 shows the protein analyses of the blood sera of the foals used in this study and of the colostrum ingested. The table indicates that the euglobulin fraction of the serum proteins is either absent or is present in very small quantities in new-born foals and that the pseudoglobulin I fraction is present in only small quantities.

Results from the protein analyses of the blood sera from the 4 foals which were allowed to suckle their dams show that there was in the serum of each of foals 1, 2, and 3, after the ingestion of colostrum, an increase in euglobulin, a very marked increase in pseudoglobulin I, and likewise an increase in total globulins. Further, a comparison of data on the blood-protein fractions for foals 1 and 2 and on the concentration of globulins in the colostrum ingested by each of them suggests that the concentration of globulins in the colostrum ingested was probably a factor in the amount of increase in the serum globulins following such ingestion. In the case of foal 4, there was an increase in euglobulin, but the pseudoglobulin I fraction was not significantly changed and a decrease occurred in both pseudoglobulin II and total globulins. Foals 1 and 2 were observed to suckle their dams during the first 24 hours after birth; each of their dams apparently had a generous supply of colostrum. However, information in regard either to the quantity or to the composition of the colostrum supplied by the dams of foals 3 and 4 is lacking. These last two foals were inconveniently located for observation of frequency of suckling, and their owner objected to any handling of their dams such as would be necessary in getting colostrum samples.

Foals 5, 7, and 9 were fed measured amounts of their dams' colostrum. Protein fractions were determined on a sample from each lot or quantity of colostrum from which the individual feedings of measured amounts were taken. From these data the total quantities of colostrum globulins ingested by each foal during the first 8 hours after birth were calculated. The protein analyses on the colostrum are shown in the table; the calculated amounts of colostrum globulins ingested are given in footnotes 4, 5, and 6.

Although the data given in the table for these foals indicate that the increases which occurred in total serum globulins within 12 hours after birth were not directly proportional to the amounts of total colostrum globulins ingested, it appears that the greater the quantity of colostrum globulins ingested by these foals, the larger the accumulation of euglobulin and of pseudoglobulin I in the serum.

TABLE 1.—Protein analyses of blood sera from foals and of the colostrum ingested by them

[Results are expressed as grams of nitrogen per 100 cc of original sample]

FOALS ALLOWED TO SUCKLE THEIR DAMS

Product analyzed and source	Time after delivery of foal		Globulin				Casein	Al-bu-min	Non-protein nitrogen	Total nitrogen
			Euglobulin	Pseudo-globulin I	Pseudo-globulin II	Total				
	Days	Hours								
Blood serum from foal 1 ¹ -----	2	0	0.010	0.046	0.065	0.121	-----	0.465	0.056	0.642
			.090	.292	.172	.554	-----	.327	.065	.946
		1	1.259	1.510		2.769	0.748	.556	.042	4.115
Colostrum from dam of foal 1 ¹ -----		12	.288	.555		.843	.502	.323	.080	1.748
		36							.121	.642
		0	.000	.024	.137	.161	-----	.458	.042	.661
Blood serum from foal 2 ¹ -----		24	.066	.142	.065	.273	-----	.396	.047	.716
	7		.096	.087	.088	.271	-----	.338	.053	.662
		1	.874	.928		1.802	.948	.277	.115	3.142
Colostrum from dam of foal 2-----		12	.141	.182		.323	.376	.192	.056	.947
		24	.066	.000		.066	.290	.103	.053	.512
		0	.021	.042	.051	.114	-----	.528	.032	.674
Blood serum from foal 3 ¹ -----		20	.038	.135	.111	.284	-----	.415	.039	.738
		44	.033	.133	.100	.266	-----	.423	.045	.734
Blood serum from foal 4 ¹ -----		0	.007	.046	.138	.191	-----	.449	.039	.679
		24	.051	.050	.072	.173	-----	.462	.044	.679

FOALS FED MEASURED QUANTITIES OF COLOSTRUM OF THEIR DAMS

Blood serum from foal 5 ¹ -----		0	0.000	0.029	0.068	0.097	-----	0.462	0.044	0.603
		12	.058	.094	.087	.239	-----	.391	.044	.674
Colostrum from dam of foal 5-----		1	.393	.351		.744	0.438	.299	.059	1.540
		6	.154	.067		.221	.098			.728
		0	.000	.028	.059	.087	-----	.552	.040	.679
Blood serum from foal 7 ¹ -----		12	.115	.227	.095	.437	-----	.449	.031	.967
		24	.037	.229	.069	.335	-----	.471	.034	.840
		1	1.128	.964		2.092	1.050	.453	.071	3.666
Colostrum from dam of foal 7-----		2	1.110	.894		1.974	1.056	.580	.050	3.660
		4	1.015	.458		1.473	1.495	.635	.047	3.650
		6				.878	.278	.038	.050	1.844
		0	.002	.054	.179	.235	-----	.558	.034	.827
Blood serum from foal 9 ¹ -----		12	.118	.108	.127	.353	-----	.515	.034	.902
		24	.037	.204	.131	.372	-----	.477	.031	.880
Colostrum from dam of foal 9-----		1	.490	.452		.942	.840	.468	.046	2.297
		3	.468	.375		.843	.408	.487	.043	1.871

FOALS RECEIVING MILK (NO COLOSTRUM)⁷

Blood serum from foal 15-----		0	0.003	0.019	0.068	0.090	-----	0.559	0.042	0.691
		12	.021	.028	.062	.099	-----	.522	.036	.657
		33	.032	.040	.051	.113	-----	.414	.039	.566
Blood serum from foal 16-----		0			.149	.230	-----	.497	.028	.755
		12	.000			.208	-----	.505	.022	.735
		36			.159	.182	-----	.534	.028	.744
Blood serum from foal 17-----		0	.013	.024	.068	.124	-----	.412	.035	.571
		24	.017	.007	.139	.163	-----	.379	.036	.570

¹ Suckled vigorously within 3 hours after delivery.² Suckled infrequently and listlessly during first 24 hours.³ Not observed between times of taking blood samples.⁴ Received, during the first 8 hours after birth, 10.3 g of colostrum globulin nitrogen.⁵ Received, during the first 8 hours after birth, 20.9 g of colostrum globulin nitrogen.⁶ Received, during the first 8 hours after birth, 17.1 g of colostrum globulin nitrogen.⁷ The milk fed was a mixture of dried cow's milk, sugar, and water, which had a total protein concentration approximating that of fresh mare's milk.

Table 1 also indicates that for foals 7 and 9 the large increases obtained for the euglobulin fraction within a few hours after the ingestion of colostrum are very transient. In each instance, at 24 hours after birth, the euglobulin fraction had dropped to a considerably lower value, which, however, was greater than that observed in

the serum of the new-born foals. On the other hand, the pseudoglobulin I fraction which, within a few hours after the ingestion of colostrum, had shown an increase comparable to that occurring in the euglobulin fraction, showed at 24 hours after birth, no such attendant decrease.

No consistency in the fluctuations in the pseudoglobulin II fraction was observed in the foals which received colostrum.

That such increases in serum globulins as occurred in foals 5, 7, and 9 do not occur when foals are fed, instead of colostrum, a product extremely poor in globulins, such as cow's milk, is indicated by the data in table 1. Foals 15, 16, and 17 were fed a mixture of dried cow's milk, sugar, and water which was comparable in total protein concentration to fresh cow's milk. In no case was there a significant increase in serum globulins. These foals succumbed within 2 weeks to one or more of the infections commonly associated with the condition known as "navel and joint ill" in foals. Foal 4 also displayed at 2 weeks of age the swollen joints, acute lameness, and marked lassitude associated with this condition. This last foal was the only one of those left with their dams in which no marked increase in pseudoglobulin I and in total globulins was observed.

KIDS

Protein analyses of sera from three kids, presented in table 2, indicate that serum euglobulin was present in very slight quantities in these animals at birth and that pseudoglobulin I was present in but little larger quantities.

TABLE 2.—*Protein analyses of blood sera from kids and of the colostrum ingested by one of them*

[Results are expressed as grams of nitrogen per 100 cc of original sample]

Product analyzed and source	Time after delivery of kid		Globulin				Casein	Albumin	Non-protein nitrogen	Total nitrogen
			Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Total				
Blood serum from kid 1, fed colostrum from dam ¹	Days	Hours								
	-----	1	0.006	0.093	0.177	0.276	-----	0.299	0.023	0.598
	-----	24	.211	.232	.197	.640	-----	.213	.052	.905
	3	-----	.135	.164	.254	.553	-----	.180	.067	.750
	6	-----	.174	.157	.185	.516	-----	.162	.088	.766
Colostrum from dam of kid 1	13	-----	.105	.136	.165	.406	-----	.340	.066	.812
	-----	1	.530	0.754	1.284	0.992	-----	.108	.176	2.560
	-----	16	.141	.146	.287	.539	-----	.062	.112	1.000
	-----	24	.048	.060	.108	.500	-----	.077	.084	.789
	-----	0	.003	.075	.076	.154	-----	.410	.087	.651
Blood serum from kid 2, fed 120 cc sheep colostrum and only goat's milk after third hour ²	-----	24	.042	.084	.041	.167	-----	.390	.068	.625
	3	-----	.033	.085	.043	.161	-----	.374	.051	.586
	6	-----	.023	.037	.115	.175	-----	.337	.051	.563
	20	-----	.076	.025	.109	.210	-----	.539	.043	.792
	-----	0	.024	.039	.114	.177	-----	.405	.095	.677
Blood serum from kid 3 allowed to suckle dam	-----	24	.163	.341	.032	.536	-----	.283	.103	.922
	3	-----	.125	.173	.090	.388	-----	.316	.059	.763
	6	-----	.084	.146	.117	.347	-----	.375	.059	.781
	20	-----	.063	.111	.102	.276	-----	.468	.059	.803

¹ Received during first 24 hours 15.2 g total nitrogen of which 7.7 g were from colostrum globulins.

² Received during first 24 hours 6.6 g total nitrogen of which 2 g were from colostrum globulins.

Twenty-four hours after the ingestion of the first goat colostrum by kids 1 and 3, the sera from these kids contained relatively large quantities of euglobulin and greatly increased quantities of pseudoglobulin I. This sharp rise in these two fractions was followed by a

gradual decrease in the concentration of both protein fractions in the blood of the animals during the time each was observed, which was 13 days in one case and 20 days in the other.

Although kid 2 received no goat colostrum, it was fed a small quantity of ewe's colostrum. The increases in euglobulin, pseudoglobulin I, and total globulins at the end of 24 hours may or may not be significant of absorption of globulin from the ewe's colostrum. The quantity of ewe's colostrum available for feeding this animal was insufficient for conclusive results in regard to the possibility of absorption of heterologous globulins as detected by the technique used. There are, however, observations in this laboratory that the new-born of one species can absorb certain proteins of another species in considerable quantities. This absorption of foreign protein has already been demonstrated by immunological reactions. Results obtained by the writer indicate that under certain conditions the absorption may be an extensive gross absorption demonstrable by chemical analysis.

LAMBS

Table 3 shows the protein analyses of the blood sera of the lambs used in this study, and of the colostrum ingested.

The blood of the four new-born lambs that were allowed to suckle their dams contained no significant quantity of euglobulin and only very small quantities of pseudoglobulin I. Within 24 hours after birth, however, large quantities of euglobulin appeared, and there was a sharp increase in the pseudoglobulin I fraction. These increases were followed by a subsequent gradual decrease in the same fractions and in total globulins during the time the lambs were studied.

Of the two lambs which were fed cow's milk instead of colostrum, lamb 39-JS was fed a 20-percent dried-milk mixture with a total protein content comparable to that of ewe's milk. Analyses of the blood serum indicated, at the end of the first 18 hours, a slight decrease in total globulin and in total nitrogen with approximately no change in the globulin-albumin ratio. Lamb 37-JH was fed a 56-percent dried-milk mixture comparable to colostrum in total protein concentration. The blood serum of this animal, after 26 hours, showed a slight increase in globulins and a small increase in the globulin-albumin ratio, which, however, was not comparable to the great increase in the globulin-albumin ratios in those animals which received colostrum.

TABLE 3.—Protein analyses of blood sera from lambs and of colostrum ingested by them

[Results are expressed as grams of nitrogen per 100 cc of original sample]

LAMBS ALLOWED TO SUCKLE THEIR DAMS

Product analyzed and source	Time after delivery of lamb		Globulin				Casein	Albumin	Non-protein nitrogen	Total nitrogen
			Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Total				
Blood serum from lamb 32-I	Days	Hours								
	0	24	0.000	0.047	0.082	0.109	-----	0.441	0.067	0.617
	2		.285	.233	.083	.601	-----	.267	.074	.941
	6		.149	.140	.084	.373	-----	.375	.067	.832
Colostrum from ewe 25-A, dam of lamb 32-I.	10		.068	.125	.087	.280	-----	.447	.085	.812
	0.5		.718	0.472		1.190	1.117	.553	.107	2.967
	24		.243	.172		.415	.769	.257	.109	1.550
	0		.003	.064	.092	.159		.318	.079	.556
Blood serum from lamb 39-I.	24		.316	.218	.123	.657		.201	.084	1.002
	2		.201	.250	.077	.528		.292	.091	.911
	4		.167	.233	.110	.510		.349	.051	.910
	35		.085	.107	.138	.330		.486	.044	.860
Colostrum from ewe 18-E, dam of lamb 39-I.	0.5		1.738	.525		2.263	1.205	.282	.307	4.067
	24		.092	.099		.191	.744	.155	.186	1.276
	0		.000	.054	.152	.206		.406	.040	.652
	24		.322	.248	.051	.621		.385	.071	1.077
Blood serum from lamb 47-I	2		.295	.202	.099	.596		.381	.055	1.032
	4		.209	.198	.071	.478		.393	.075	.946
	10		.146	.147	.102	.395		.378	.084	.857
	38		.080	.111	.208	.399		.328	.062	.789
Colostrum from ewe 23-B, dam of lamb 47-I.	0.5		.817	.756		1.573	1.626	.396	.038	3.633
	7		.595	.199		.794	1.094	.297	.239	2.424
	24		.102	.151		.253	.611	.036	.084	.984
	0		.000	.013	.231	.244				.741
Blood serum from lamb 53-I	24		.267	.220	.062	.549		.363	.096	1.008
	2		.190	.179	.111	.480		.369	.084	.933
	5		.153	.180	.112	.445				.883
	11		.130	.143	.096	.369		.440	.065	.874
Colostrum from ewe 17-G, dam of lamb 53-I.	18		.093	.131	.103	.327		.459	.070	.856
	0.5		.980	.730		1.710	1.556	.540	.256	4.062
	2		.476	.003		.479	.676	.101	.136	1.392

LAMBS RECEIVING MILK (NO COLOSTRUM)

Blood serum from lamb 39-JS. ¹	1	0.006	0.084	0.098	0.188	-----	0.457	0.055	0.700
Milk mixture fed to lamb 39-JS.	18	.004	.047	.114	.165	-----	.388	.041	.594
						-----			.860
Blood serum from lamb 37-JH. ²	1	.043	.018	.086	.147	-----	.396	.054	.697
	26	.052	.020	.107	.179	-----	.348	.082	.609
Milk mixture fed to lamb 37-JH.	47	.012	.064	.123	.199	-----	.425	.032	.656
						-----			2.400

¹ Received during first 12 hours after birth 3.42 g total nitrogen in a 20-percent mixture of dried cow's milk in water.² Received during first 20 hours after birth 20.8 g total nitrogen in a 56-percent mixture of dried cow's milk in water.

PIGS

Table 4 presents the results of protein analyses of sera from four pigs. Sera from these animals at birth contained very small quantities of the globulin fractions. In pig 2, which ingested only milk and which was bled shortly after birth and again 2 days later, there was no marked increase in any of these fractions. All the animals, except pig 13, died on the third day. Within 24 hours after the birth of this pig the total globulins in the serum were about six times as great as those in the serum of new-born animals. This was due largely to the increase in euglobulin. There was a decrease in this large proportion of euglobulin during the 12 days in which the pig was observed.

TABLE 4.—*Protein analyses of blood sera from 4 pigs of the same litter, and of colostrum from the dam*

[Results are expressed in grams of nitrogen per 100 cc of original sample]

Product analyzed and source	Time after delivery of pig		Globulin				Casein	Albumin	Non-protein nitrogen	Total nitrogen
			Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Total				
Blood serum from pigs placed with sow farrowed 8 days previously:	<i>Days</i>	<i>Hours</i>								
Pig 2.....	2	0.5	0.031	0.040	0.062	0.133	-----	-----	-----	0.460
Pig 4.....	24		.035	.039	.103	.177	-----	0.191	0.121	.489
			.013	.080	.081	.174	-----	.162	.149	.485
Blood serum from pigs allowed to suckle dam:										
Pig 1.....		.5	.017	.061	.060	.138	-----	.123	.188	.449
Pig 13.....	24		.607	.177	.072	.856	-----	.207	.106	1.169
	4		.285	.199	.087	.581	-----	-----	-----	.923
	12		.168	.201	.097	.466	-----	.484	.052	1.002
Colostrum from dam suckled by pig 13.....	1		1.680	0.541		2.221	1.234	.457	.242	4.154

The serum of the new-born pigs differed from the serum of the new-born of the other species in its remarkably high nonprotein nitrogen content. Results obtained from the pigs during the first 2 days after birth and before the ingestion of colostrum indicated approximately 25 percent or more of total serum nitrogen to be in the form of non-protein nitrogen.

CONCLUSIONS

From the data presented in this paper it is concluded that the effect of colostrum ingestion on the serum proteins of young foals, kids, lambs, and pigs is similar to that observed by Howe in calves, in that after the ingestion of colostrum there is a striking rise in the concentration of total nitrogen, which rise is occasioned by large increases in the euglobulin and pseudoglobulin I fractions. The author further concludes from the work here reported that young foals, kids, lambs, and pigs which are fed homologous colostrum during the first 24 to 48 hours after birth absorb, from the colostrum ingested, euglobulin and pseudoglobulin I. It seems highly probable that absorption of immune substances takes place largely through absorption of the globulins with which they are so closely associated.

In a project concerned with the use of milk substitutes and supplements in the feeding of orphan farm animals, it appears that

any program for feeding these new-born animals should include either homologous colostrum or some substitute from which the new-born animals may absorb either the protective substances ordinarily supplied in the colostrum, or their equivalent. There is the possibility that the absorption of certain types of globulins in which the serum is deficient at birth may enable the young animal to elaborate its own antibodies.

SUMMARY

Results of studies of the protein fractions of sera from 10 foals, 3 kids, 6 lambs, and 4 pigs indicate that in each of the four species, the serum of the new-born animal is deficient in the euglobulin fraction of the serum proteins. In every instance pseudoglobulin I was present but only in very small quantities.

In those animals which received no colostrum from their respective dams there was little or no increase in serum globulins during the period of observation, with the exception of one kid whose serum contained increased globulins on the twentieth day after birth.

In the young foals, kids, lambs, and the one pig known to have received colostrum from their respective dams, there was a marked increase in total serum nitrogen within 24 hours after birth. This change resulted from increases in euglobulin and in pseudoglobulin I in the serum. There was a decrease in these fractions in the animals studied for some days after this rise.

It has been shown in young foals that the amount of absorption of euglobulin and pseudoglobulin I is related to the quantity of colostrum globulins ingested.

LITERATURE CITED

- (1) BOYD, G. L.
1922. THE VALUE OF COLOSTRUM TO THE NEWBORN. *Canada Med. Assoc. Jour.* 12: 724-725.
- (2) BRAUN, H., HOFMEIER, K., and HOLZHAUSEN, G. v.
1929. DIE VERERBUNGSFRAGE IN DER LEHRE VON IMMUNITÄT GEGEN INFektionsKRANKHEITEN. In Kollé, W., and Wassermann, A. v., eds., *Handbuch der Pathogenen Mikroorganismen*. Aufl. 3, Bd. 1, lfg. 29, pp. [1109]-1146, illus. Jena.
- (3) CLARK, E. P., and COLLIP, J. B.
1926. A PROCEDURE FOR THE DETERMINATION OF UREA IN FOLIN-WU BLOOD FILTRATES BY THE AUTOCLAVE METHOD. *Jour. Biol. Chem.* 67: 621-627, illus.
- (4) EHRLICH, P.
1892. UEBER IMMUNITÄT DURCH VERERBUNG UND SÄUGUNG. *Ztschr. Hyg. u. Infektionskrankh.* 12: [183]-203.
- (5) HOWE, P. E.
1921. THE DETERMINATION OF PROTEINS IN BLOOD—A MICROMETHOD. *Jour. Biol. Chem.* 49: 109-113.
- (6) ———
1921. AN EFFECT OF THE INGESTION OF COLOSTRUM UPON THE COMPOSITION OF THE BLOOD OF NEW-BORN CALVES. *Jour. Biol. Chem.* 49: 115-118.
- (7) ———
1922. THE DIFFERENTIAL PRECIPITATION OF THE PROTEINS OF COLOSTRUM AND A METHOD FOR THE DETERMINATION OF THE PROTEINS OF COLOSTRUM. *Jour. Biol. Chem.* 52: 51-68.
- (8) ———
1923. THE RELATIVE PRECIPITATING CAPACITY OF CERTAIN SALTS WHEN APPLIED TO BLOOD SERUM OR PLASMA AND THE INFLUENCE OF THE CATION IN THE PRECIPITATION OF PROTEINS. *Jour. Biol. Chem.* 57: 241-254.

-
- (9) KUTTNER, A., and RATNER, B.
1923. THE IMPORTANCE OF COLOSTRUM TO THE NEW-BORN INFANT. *Amer. Jour. Diseases Children* 25: [413]-434.
- (10) LEWIS, J. H., and WELLS, H. G.
1922. THE FUNCTION OF THE COLOSTRUM. *Jour. Amer. Med. Assoc.* 78: 863-865.
- (11) LITTLE, R. B., and ORCUTT, M. L.
1922. THE TRANSMISSION OF AGGLUTININS OF *BACILLUS ABORTUS* FROM COW TO CALF IN THE COLOSTRUM. *Jour. Expt. Med.* 35: 161-171.
- (12) MASON, J. H., DALLING, T., and GORDON, W. S.
1930. TRANSMISSION OF MATERNAL IMMUNITY. *Jour. Path. and Bact.* 33: 783-797, illus.
- (13) NELSON, J. B.
1932. THE MATERNAL TRANSMISSION OF VACCINAL IMMUNITY IN SWINE. *Jour. Expt. Med.* 56: 835-840, illus.
- (14) ORCUTT, M. L., and HOWE, P. E.
1922. THE RELATION BETWEEN THE ACCUMULATION OF GLOBULINS AND THE APPEARANCE OF AGGLUTININS IN THE BLOOD OF NEW-BORN CALVES. *Jour. Expt. Med.* 36: 291-308.
- (15) PREGI, F.
1930. QUANTITATIVE ORGANIC MICROANALYSIS. 2d English Ed., Transl. from 3d rev. and enl. German ed. by E. Fyleman . . . 237 pp., illus. London.
- (16) SMITH, T., and LITTLE, R. B.
1922. THE SIGNIFICANCE OF COLOSTRUM TO THE NEW-BORN CALF. *Jour. Expt. Med.* 36: 181-198.
- (17) ——— and LITTLE, R. B.
1922. COW SERUM AS A SUBSTITUTE FOR COLOSTRUM IN NEW-BORN CALVES. *Jour. Expt. Med.* 36: 453-468.
- (18) ——— and LITTLE, R. B.
1923. THE ABSORPTION OF SPECIFIC AGGLUTININS IN HOMOLOGOUS SERUM FED TO CALVES DURING THE EARLY HOURS OF LIFE. *Jour. Expt. Med.* 37: 671-683.
- (19) ——— and LITTLE, R. B.
1930. THE IMMUNOLOGICAL SIGNIFICANCE OF COLOSTRUM. II. THE INITIAL FEEDING OF SERUM FROM NORMAL COWS AND COWS IMMUNIZED TOWARD *B. COLI* IN PLACE OF COLOSTRUM. *Jour. Expt. Med.* 51: 483-492.

A STUDY OF THE CAUSE OF VARIABILITY IN RESPONSE OF BARLEY LOOSE SMUT TO CONTROL THROUGH SEED TREATMENT WITH SURFACE DISINFECTANTS¹

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INTRODUCTION

The present investigation was undertaken to discover the factor or factors involved in the variability of response of barley loose smut to control through treatment of the seed with surface disinfectants.

Previous to Johnson's report in 1914 (13)² it seemingly had been well established that the fungus causing loose smut (*Ustilago nuda* (Jens.) Kell. and Sw.) in barley (*Hordeum vulgare* L.) was controllable through seed disinfection only by the application of a prolonged and deeply penetrating treatment like the modified hot-water method of Freeman and Johnson (5). Since 1914, however, and especially in the past 10 years, it has been repeatedly demonstrated in the United States that the treatment of naturally inoculated barley seed with surface disinfectants such as formaldehyde solution and certain organic mercury solutions and dusts may completely control loose smut, reduce it, or fail to effect any appreciable reduction (16, 20, 22, 23, 27, 31, 32). In the absence of a knowledge of the factor or factors involved in the variable responses of barley loose smut to control through seed treatment with surface disinfectants, it has not seemed advisable, however, to recommend these easily applied remedies.

The modified hot-water bath has seemed the only certain method for controlling loose smut in barley through seed treatment. This treatment is so difficult to apply, however, and frequently so injurious to germination that it rarely has been used by farmers. Therefore, no practical measure for controlling loose smut of barley through seed treatment has been available. The results of studies establishing the factor responsible for the varying effectiveness of seed surface disinfectants in barley loose smut control are presented in the following pages.

REVIEW OF PREVIOUS INVESTIGATIONS

A detailed review of early investigations of the life history and control of *Ustilago nuda* has been given by Tisdale and Tapke (30). Since its description in 1889 (15) this fungus has been considered the sole pathogene causing loose smut in barley. It was shown that the loosely held spores from smutty heads are blown, washed, or carried by insects to the flowers of normal heads when the glumes are open at blooming. Except under arid conditions, as has more recently been demonstrated (25), these spores soon germinate, producing hyphae that grow directly into and deeply within the developing seeds. The fungus remains dormant within the seeds until they are

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² Reference is made by number (*italic*) to Literature Cited, p. 506.

sown; it revives as the seeds germinate and grows up through the young plants, causing the smutty heads. Two other items having an important bearing on the present investigation also should be noted. It was definitely established by 1914 that (1) *U. nuda* is strictly a floral-infecting smut and is incapable of producing smutted plants from mature barley seed inoculated with smut spores (1, 2, 5, 10, 21), and (2) the deeply embedded fungus in infected seed from inoculated flowers is not controlled by treating the seed with surface disinfectants. A prolonged and deeply penetrating seed treatment like the modified hot-water method of Freeman and Johnson (5) is necessary to effect control. Reports emanating from countries other than the United States apparently still are in agreement with the facts of life history and control as just cited.

Beginning with Johnson's report in 1914 (13), however, the findings of investigators in this country frequently have failed to parallel those of the early workers. Relative to infection, Tisdale and Tapke (30) in 1924 obtained from 52.5 to 100 percent of smutted plants in the varieties Alaska, Greece, Han River, Texas Winter, and Wisconsin Winter as a result of applying loose smut spores to mature seed from which the hulls had been removed. A few years later Tisdale and Griffiths (29) used 32 different collections of loose smut to inoculate dehulled seed of Tennessee Winter and Hannchen barley. Most of the collections produced high percentages of smutted plants in Tennessee Winter or in both of the varieties. Six collections, however, were unable to produce smutted heads in either of the varieties.

Abundant proof has been furnished in the past 10 years that, at least under certain conditions, loose smut in barley may be controlled through treatment of the seed with surface disinfectants. Tisdale et al. (31), using naturally inoculated seed of Cusado, Greece, Han River, Tennessee Winter, Texas Winter, and Wisconsin Winter barleys, concluded that "formaldehyde, which has been recommended for the control of covered smut of barley, controlled loose smut better than it controlled covered smut." In a later test Tisdale et al. (32), obtained excellent control of loose smut in Cusado, Orel, Tennessee Winter, and Wisconsin Winter by treating the naturally inoculated seed with solutions of various organic mercury compounds for 15 minutes to 1 hour. Similar results were obtained by Taylor and Zehner (27) in a 5-year test of Tennessee Winter and Wisconsin Winter grown from naturally inoculated seed immersed in a 0.3-percent solution of Semesan for 1 hour. In tests with dust fungicides, Leukel (20) obtained results indicating that control of barley loose smut through seed treatment with surface disinfectants is a function either of the host variety or of the different loose smuts associated with the different varieties. For example, through treatment of naturally inoculated seed with Ceresan or Semesan dust in 1928-29 and again in 1929-30, he obtained excellent control in Tennessee Winter and Wisconsin Winter, approximately 50 percent control in Orel, and only a negligible reduction of loose smut in Esaw. This was the state of knowledge forming the background of the present study.

METHODS OF EXPERIMENTATION

The experimental procedure, as shown later, was concerned chiefly with (1) floral and seed inoculations of barley with various collections of loose smut and (2) treatment with surface disinfectants of the seed

from hand-inoculated flowers. It was found necessary, however, to develop first a special technic in order to avoid serious injury to seed viability from the inoculation of barley flowers as well as from the application of surface disinfectants to the seed from the inoculated flowers.

Freeman and Johnson (5) have shown that the period during which barley flowers are susceptible to infection by *Ustilago nuda* extends from the time when the pollen is still immature until the fertilized ovary has attained approximately one-third of its mature size. In the present study the floral inoculations were made well within the limits of this period. In the early studies inoculation was performed by using forceps to open the glumes (when closed flowers were inoculated) and to place the smut on the stigma. The smut was conveniently carried in a capsule placed in a clamp on a thumb ring as shown in figure 1. The device is an adaptation of the handy pollen carrier devised by Leighty and Sando (19). Previous experience with this method had proved highly satisfactory in the inoculation of wheat flowers with *U. tritici* (24). It was found, however, in the case of barley that the method was slow and tedious because of the difficulty in opening the interlocking glumes, and that seed from flowers so inoculated is shriveled, low in vitality, and highly susceptible to injury when treated with Ceresan dust. Zeiner (33) also employed a floral-inoculation method involving spreading of the glumes, and reported similar results relative to low seed vitality. Undoubtedly barley is more susceptible to injury from spreading the glumes than is wheat. The inoculation of barley flowers without spreading of the glumes may be readily accomplished by clipping the upper portion of the spikelet, thereby bringing the stigma into view. This method is objectionable because the removal of the awns adversely affects kernel development, as shown by Harlan and Anthony (7).

A method of inoculation was evolved, however, that produced satisfactory results. It consists in dipping the pointed tips of forceps in the spore dust contained in the capsule of the thumb ring (fig. 1), then using the tips to pierce the central portion of one of the glumes and to insert inoculum on the stigma. By letting the inserted forceps expand slightly, the glume aperture is widened and the stigma is brought into view. If the tips of the forceps are spread slightly when placed in the spore dust and then pressed together, a considerable amount of inoculum is squeezed into the corrugations of the inner faces of the tips. This may be gradually deposited on the stigmas of from 5 to 10 successive flowers and results in a marked speeding of the work, reducing the time of inoculating a flower to but little more than that consumed in puncturing a glume. Seed developed from flowers thus inoculated was as plump and viable as that from uninoculated flowers. It was found, however, that the seed from inoculated flowers still was susceptible to injury when treated with Ceresan but was apparently wholly uninjured when immersed for 60 to 90 minutes in a formaldehyde solution containing 1 part of a 37- to 40-percent formaldehyde solution in 320 parts of water, followed by a thorough rinse in water. As noted later, there was need for an effective and noninjurious medium to disinfect the surface and near-surface parts of some of the seed from inoculated flowers. The modi-

fied method of inoculation and the formaldehyde seed treatment just described were adopted after the first experiment. (See table 1.)

In the use of the modified inoculation method it is distinctly advantageous to sift the smut through a 40-mesh sieve after its removal from the head. This process removes extraneous material and facilitates the handling of the inoculum. It is also an aid in determining the exact color of the mass of smut spores, the importance of which is shown later.

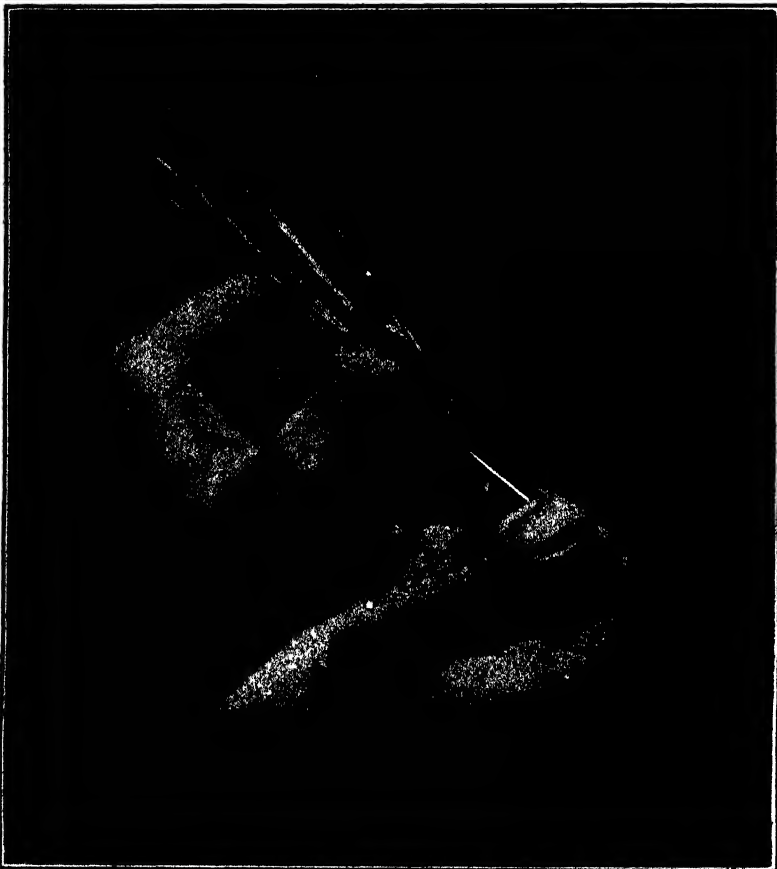


FIGURE 1.—Handy device for use in inoculating barley flowers with loose smut. It consists of a thumb ring with a side clamp to hold a capsule containing the inoculum. The knob on top of the ring holds the upper part of the capsule when the device is in use. (Adaptation of a pollen carrier devised by Leighty and Sando.)

In addition to speed and apparent lack of interference with normal seed development, the above-described method has the following advantages. (1) Different collections of smut may be conveniently carried to the field in individual capsules placed in envelopes appropriately labeled. (In making the change from one collection of inoculum to another, only a few seconds are required to replace a capsule in the clamp of the thumb ring and to disinfect the forceps by flaming after immersion in alcohol.) (2) The insertion of inoculum only on the sticky stigma reduces to a minimum the chances of

contaminating flowers of adjacent heads with errant spores from the collection in use. (3) The small amounts of inoculum usually received from collectors may be used to advantage because none of the smut is wasted.

EXPERIMENTAL RESULTS

A test of two lots of barley in 1929 had shown that treatment of the naturally inoculated seed with Ceresan dust controlled the loose smut in a lot of Wisconsin Pedigree No. 5 from Wisconsin, but only slightly reduced it in a lot of Featherston from New York. These two varieties and the loose smuts collected from them were used in the following experiment conducted at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., to determine whether success or failure in obtaining control through surface disinfection of the seed might be traceable to the smuts or to the barley varieties concerned. In April 1930, flowers and seeds of the Wisconsin Pedigree No. 5 in various stages of development, as shown in table 1, were inoculated by hand with smut from each of the two varieties. Featherston was similarly inoculated. The plants bearing the flowers that were inoculated were grown from seed treated by the modified hot-water method and were smut-free and grown in a greenhouse that contained no smutted plants. The loose smut used to inoculate the flowers was fresh material collected from plants grown in another greenhouse from untreated seed of the same two lots. The seed collected from the inoculated heads was then sown in a greenhouse in the autumn of 1930. The results shown in table 1 were obtained in the spring of 1931.

TABLE 1.—*Control of loose smut in barley as influenced by the varietal source of inoculum, variety inoculated, stage of anthesis at inoculation, and treatment of the seed with Ceresan in 1930-31*

Collection no. of inoculum	Variety from which inoculum was obtained	Variety inoculated ¹	Stage of anthesis at inoculation	Seed (from inoculated flowers) untreated or treated with Ceresan	Total number of plants in 1931	Smutted plants	
						Number	Percent
100	Featherston	Featherston	Pollen green	Untreated	54	11	20.4
				Treated	25	6	21.4
			Pollen ripe, flowers in bloom	Untreated	72	24	33.3
				Treated	35	12	33.3
		Wisconsin Pedigree No. 5	Pollen shed, ovaries $\frac{1}{4}$ - $\frac{1}{2}$ developed	Untreated	82	16	19.5
				Treated	54	4	7.4
			Pollen green	Untreated	72	13	16.7
				Treated	20	3	15.0
		do.	Pollen ripe, flowers in bloom	Untreated	81	17	21.0
				Treated	18	3	16.7
			Pollen shed, ovaries $\frac{1}{4}$ - $\frac{1}{2}$ developed	Untreated	67	5	7.5
				Treated	34	1	2.9
101	{ Wisconsin Pedigree No. 5.	Featherston	Pollen green	Untreated	56	0	.0
				Treated	24	0	.0
			Pollen ripe, flowers in bloom	Untreated	40	0	.0
				Treated	18	0	.0
		do.	Pollen shed, ovaries $\frac{1}{4}$ - $\frac{1}{2}$ developed	Untreated	62	0	.0
				Treated	43	0	.0
		Wisconsin Pedigree No. 5	Pollen green	Untreated	68	63	92.8
				Treated	24	0	.0
			Pollen ripe, flowers in bloom	Untreated	95	89	93.7
				Treated	35	0	.0
		do.	Pollen shed, ovaries $\frac{1}{4}$ - $\frac{1}{2}$ developed	Untreated	74	69	93.2
				Treated	44	0	.0

¹ 97 control plants of Featherston and 94 of Wisconsin Pedigree No. 5 were smut-free. They were grown from untreated seed of flowers that were not inoculated.

The data of table 1 point to the conclusion that the smut rather than the host variety determines whether or not loose smut in barley may be controlled through surface disinfection of seed from inoculated flowers. Featherston proved immune to the Wisconsin Pedigree No. 5 smut (collection no. 101). Wisconsin Pedigree No. 5, however, was highly susceptible to its own smut (collection no. 101), producing over 90 percent of smutted plants when the seed from inoculated flowers was untreated; when the seed was treated with Ceresan dust, complete control of loose smut was obtained. The different stages of anthesis at the time of floral inoculation with this smut exerted no influence on the degree of smuttedness in plants from untreated seed or on the effectiveness of seed treatment in the control of smut in plants from treated seed. The Ceresan seed treatment was ineffective on both varieties, however, when applied to seed from flowers inoculated in the bloom and prebloom stages with the Featherston smut (collection no. 100). Inoculation of the flowers with this smut shortly after blooming, i. e., when the ovaries were beginning to enlarge, resulted in a decrease in the percentage of smutted plants and an increase in the effectiveness of seed treatment. These results with the Featherston smut with respect to stage of anthesis at inoculation parallel those obtained by Freeman and Johnson (5) with *Ustilago nuda*.

In 1931 and 1932 experiments were conducted in the spring to determine the ability of the Featherston and Wisconsin Pedigree No. 5 smuts to produce smutted plants as a result of applying smut spores to mature barley seed. In 1931 seed of Alpha, Featherston, and Wisconsin Pedigree No. 5 barleys was inoculated with each of the two smuts collected from its respective variety (collections nos. 100 and 101). In 1932 these smuts again were used, but in most instances, as shown in table 2, they were collected from varieties other than those from which they were collected in 1931. This was done to determine any possible influence of the host variety on the pathogenicity of the causal fungus. Results obtained by Tisdale and Griffiths (29) had led them to suspect a relation of this kind. In 1932, seed of the 3 varieties used in 1931 and of 7 or 8 additional varieties was inoculated with each of the smut collections (nos. 100 and 101). Also in 1932, seed of Esaw barley was inoculated with the smut (collection no. 106) that naturally occurs on and usually severely attacks this variety at the Arlington farm. Previous tests by Leukel (20) had shown that treatment of the naturally inoculated Esaw seed with Ceresan or Semesan dusts was ineffective in smut control. In both 1931 and 1932 the seed either was obtained from plants unexposed to smut or was treated by the modified hot-water method. Following removal of the hulls, the seed was blackened with smut spores and immediately sown in moderately moistened soil in deep flats. The soil was maintained at temperatures close to 21° C. until the seedlings emerged; then the plants in the flats were placed outdoors to complete growth. The collections of inoculum were obtained from plants grown in the greenhouse and in no case were more than 1 month old when applied to the seed. All of them proved highly viable in germination tests at the time the seed was sown. Results of the test are presented in table 2.

TABLE 2.—*Incidence of loose smut in varieties of barley as a result of inoculating mature seed with 3 loose smut collections*

Collection no. of inoculum	Variety from which inoculum was obtained in—		Varieties inoculated (inoculum applied to mature seed ¹)	Year of test	Total number of plants	Smutted plants	
	1931	1932				Number	Per-cent
100.....	Featherston.....	Alpha.....	Alpha.....	1931	89	0	0.0
		Featherston.....	Featherston.....	1931	87	0	0.0
		Wisconsin Pedigree No. 5.....	Wisconsin Pedigree No. 5.....	1931	80	0	0.0
		Alpha.....	Alpha.....	1932	78	0	0.0
		do.....	Black Barbless.....	1932	54	0	0.0
		do.....	Colsees.....	1932	34	0	0.0
		do.....	Featherston.....	1932	104	0	0.0
		do.....	Glabron.....	1932	24	0	0.0
		do.....	Hannchen.....	1932	56	0	0.0
		do.....	Nepal.....	1932	114	0	0.0
		do.....	Spartan.....	1932	44	0	0.0
		do.....	Trebl.....	1932	64	0	0.0
		do.....	Wisconsin Pedigree No. 5.....	1932	122	0	0.0
		Wisconsin Pedigree No. 5.....	Alpha.....	1932	76	0	0.0
		do.....	Featherston.....	1932	114	0	0.0
		do.....	Wisconsin Pedigree No. 5.....	1932	104	0	0.0
		Alpha.....	Alpha.....	1931	117	100	85.5
		Featherston.....	Featherston.....	1931	82	9	11.0
		Wisconsin Pedigree No. 5.....	Wisconsin Pedigree No. 5.....	1931	79	73	92.4
		Alpha.....	Alpha.....	1932	76	62	81.6
101.....	{ Wisconsin Pedigree No. 5.....	do.....	Black Barbless.....	1932	26	4	15.4
		do.....	Colsees.....	1932	12	4	33.3
		do.....	Esaw.....	1932	156	6	3.8
		do.....	Featherston.....	1932	70	12	17.1
		do.....	Glabron.....	1932	6	0	0.0
		do.....	Hannchen.....	1932	40	36	90.0
		do.....	Nepal.....	1932	58	0	0.0
		do.....	Spartan.....	1932	12	0	0.0
		do.....	Trebl.....	1932	60	60	100.0
		do.....	Wisconsin Pedigree No. 5.....	1932	112	107	95.5
106.....		Esaw.....	Esaw.....	1932	147	0	0.0

¹ Control plants from uninoculated seed of each of the varieties used in 1931 and 1932 also were grown and were unsmutted. In each year the number of control plants of each variety was equal to or slightly greater than the number of plants of the same variety grown from seed inoculated with smut collection no. 101.

The data of table 2 give further proof of fundamental differences relative to infection between the Featherston (collection no. 100) and Wisconsin Pedigree No. 5 (collection no. 101) smuts. In no case was the former able to produce smutted plants as a result of seed inoculation. The latter, on the other hand, produced smutted plants in 11 of the 14 tests involving 11 different barley varieties, the percentage of smutted plants in one case reaching 100. The Esaw (collection no. 106) smut produced results similar to those of the Featherston smut.

It is evident from the data of table 2 that the host varieties from which the smuts were collected exerted no noticeable influence on the subsequent pathogenicity of any of the smut collections.

The failure of the Featherston (collection no. 100) and Esaw (collection no. 106) smuts to produce smutted plants as a result of seed inoculation in this experiment was not due to a lack of viability or vitality. The same collections were again used several months later (in June) at Ithaca, N. Y., in floral inoculations of most of the varieties that were seed-inoculated in the foregoing experiment. The high percentages of smut in plants grown from seed from the inoculated flowers (table 4) show that the collections were viable.

Evidence leading to definite proof that two specifically distinct loose smut fungi were responsible for the results previously noted was obtained in an experiment conducted in the spring of 1932.

Seed of eight lots of naturally inoculated Alpha barley was treated for 1½ hours in a solution of 1 part formaldehyde in 320 parts of water, then thoroughly rinsed in water and sown at the Arlington farm. At the same time untreated seed of each of the lots also was sown. The lots were collected in 1931 from fields in various parts of New York in which the incidence of loose smut was relatively high. When the plants headed, it was observed, as shown in table 3, (1) that some of the smutted heads were olivaceous brown while others were dark chocolate-brown and (2) that the formaldehyde seed treatment effectively controlled the dark-brown smut but was ineffective in the control of the olivaceous-brown smut.

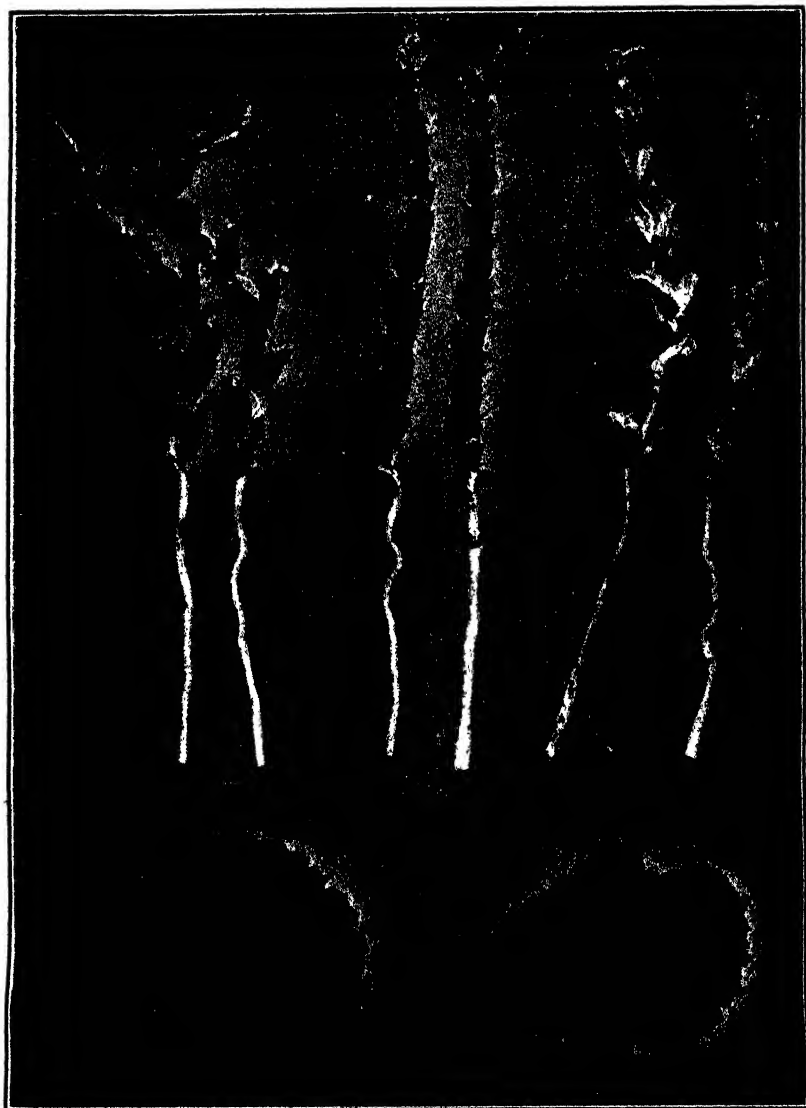
TABLE 3.—Incidence and color of loose smut heads in 8 lots of naturally inoculated Alpha barley from New York, grown from seed untreated or treated for 1½ hours in a 1 to 320 formaldehyde solution

Seed lot no.	Seed untreated or treated with formaldehyde solution	Total number of heads	Smutted heads		Color of spore mass of loose smut heads	Smut collection no.
			Number	Percent		
1	Untreated.....	332	19	5.7	Dark brown.....	118
	Treated.....	284	0	0
2	Untreated.....	222	11	5.0	Dark brown.....	119
	Treated.....	253	0	0
3	Untreated.....	244	36	14.8	Dark brown.....	120
	Treated.....	267	0	0
4	Untreated.....	194	10	5.2	Dark brown.....	121
	Treated.....	240	0	0
5	Untreated.....	198	21	10.6	Dark brown.....	122
	Treated.....	266	0	0
6	Untreated.....	189	17	9.0	12 dark brown; 5 olivaceous brown.....	123
	Treated.....	240	4	1.7	Olivaceous brown.....	126
7	Untreated.....	195	16	8.2	Dark brown.....	124
	Treated.....	228	0	0
8	Untreated.....	169	9	5.3	7 dark brown; 2 olivaceous brown.....	125
	Treated.....	219	3	1.4	Olivaceous brown.....	127

Table 3 shows that the formaldehyde treatment completely eradicated the dark-brown smut in all seed lots. In seed lots 6 and 8, in which both smuts were present, only the olivaceous-brown smut appeared in plants from treated seed. The data in table 3, therefore, give substantial support to those in table 1 in showing that the reaction of barley to seed treatment with a surface disinfectant is a function not of the host variety but of the smut or smuts carried by the seed.

Following the collection of the data presented in table 3, an inspection of the color of the smut collections used in previous experiments (tables 1 and 2) was made. It was found that the Featherston and Esaw smuts (collections nos. 100 and 106, respectively) were olivaceous brown and that the Wisconsin Pedigree No. 5 smut (collection no. 101) was a distinctly darker brown, almost black, and that these color differences in spore mass held true regardless of the last variety from which the smut was collected.

At this time, results showing further striking differences also were obtained from studies of the Featherston and Wisconsin Pedigree No. 5 smuts and from studies of other olivaceous-brown and dark-brown collections, apart from the host plants. It was found that the olivaceous-brown-spored loose smut undoubtedly was the one referred to as *Ustilago nuda* (or one of its synonyms) in the reports of Jensen (12), Kellerman and Swingle (15), Maddox (21), Brefeld (1), Brefeld and Falck (2), Hecke (8, 9), Hori (10), Broili (3),



Heads of Alpha barley infected by (A) *Ustilago nuda* and (B) *U. nigra*, showing the darker color and greater compactness of the sori of the latter. Heads of Alpha barley infected by *U. nuda* (C) and *U. nigra* (D) previous to the rupture of the peridium. At this stage the color difference in heads infected by the two loose smuts of barley is not readily distinguishable. Spore masses of *U. nuda* (E) and *U. nigra* (F), showing the darker appearance of the latter.

and Freeman and Johnson (5), because it conformed to the descriptions of these early workers in regard to size, color, and echinulate markings of spores, spore germination by production of a promycelium that does not bear sporidia, and ability to infect the host only through the flowers with subsequent hyphal penetration so deeply within the seed that the fungus is able to survive the action of seed-surface disinfectants. On the other hand, in addition to the distinguishing characters of the dark-brown loose smut previously noted, i. e., the darker color of its spores, its ability to produce seedling as well as floral infection, and its inability to survive the Ceresan dust and liquid formaldehyde treatments applied to seed from inoculated flowers, it was found that its chlamydospores are larger than those of *Ustilago nuda* and that on certain media they germinate by producing a promycelium bearing typically four lateral sporidia. Its chlamydospores, like those of *U. nuda*, are echinulate.

In an earlier paper the writer (26) proposed the name *Ustilago nigra* for this newly described darker spored loose smut. A comparison of the color of the spore masses of *U. nuda* and *U. nigra* and of the appearance of heads of Alpha barley affected with these smuts is shown in plate 1. The terms "brown" and "black" loose smuts of barley are proposed for *U. nuda* and *U. nigra*, respectively.

The foregoing studies on control of the two loose smuts of barley through surface disinfection of seed from inoculated flowers (tables 1 and 3) were restricted in regard to the number of host varieties and smut collections used. However, in 1932 and 1933, new collections were incorporated in further tests. In an experiment begun in the spring of 1932 at the Arlington farm and at Ithaca, N. Y., 22 smut collections from four States and 11 barley varieties were employed, as shown in table 4. Flowers of field-grown plants were inoculated by hand at or close to the blooming period. Seed from the inoculated flowers was collected at maturity and a part of each of the different lots was treated with formaldehyde solution (1 to 320) for 1 hour, then thoroughly rinsed in water and spread to dry for several days. Untreated and treated portions of each lot then were sown in a greenhouse at the Arlington farm in the autumn of 1932. At heading time in the spring of 1933, both olivaceous-brown (*Ustilago nuda*) and dark-brown (*U. nigra*) smut heads appeared in many of the lots. Evidently the smut collections used in the floral inoculation of such lots were mixed cultures of both species. Examination of the color and germination tests of remnant portions of the collections used in the floral inoculations confirmed this view. Soon after emergence, therefore, all of the smut heads were individually bagged. Later, when the heads were collected the olivaceous-brown and dark-brown heads from each of the various smut collections on each of the different varieties were sorted. Spores then were picked from a representative number of heads of each of the sorted lots and germinated on 2-percent potato-dextrose agar at approximately 21° C. in order to verify the species. On this medium the chlamydospores of *U. nigra* germinate and produce a promycelium bearing usually four lateral sporidia, and the chlamydospores of *U. nuda* produce a promycelium without sporidia. The species collected from smutted plants from the various lots of untreated and treated seed, as so determined, are included in the data on control presented in table 4.

TABLE 4.—Control of *Ustilago nuda* and *U. nigra* in plants of 11 varieties of barley grown from seed from flowers inoculated by hand with 1 or more of 22 collections of loose smut from various varieties and localities in the United States

[Seed untreated or immersed for 1 hour in a solution of 1 part of formaldehyde in 320 parts of water]

Smut collection no.	Variety and locality from which smut was collected in 1932		Varieties florally inoculated ¹	Seed (from inoculated flowers) untreated or treated with formaldehyde	Total number of plants	Smutted plants		Species of loose smut (<i>U. nuda</i> or <i>U. nigra</i>) collected from smutted heads in 1933
	Variety	Locality				Number	Percent	
100	Featherston	Roslyn, Va.	Alpha	Untreated	38	15	39.5	<i>U. nuda</i> .
	do.	do.	do.	Treated	24	7	29.2	Do.
	Wisconsin Pedigree No. 5.	do.	do.	Untreated	23	13	56.5	Do.
	do.	do.	do.	Treated	38	20	52.6	Do.
	Featherston	do.	Colsess	Untreated	24	12	50.0	Do.
	do.	do.	do.	Treated	36	19	52.8	Do.
	do.	do.	Featherston	Untreated	23	2	8.7	Do.
	do.	do.	do.	Treated	46	1	2.2	Do.
	Wisconsin Pedigree No. 5.	do.	do.	Untreated	23	7	30.4	Do.
	do.	do.	do.	Treated	41	18	43.9	Do.
	Featherston	do.	Glabron	Untreated	16	2	12.5	Do.
	do.	do.	do.	Treated	36	5	13.9	Do.
	do.	do.	Hannchen	Untreated	24	23	95.8	Do.
	do.	do.	do.	Treated	36	16	44.4	Do.
	do.	do.	Nepal	Untreated	9	4	44.4	Do.
	do.	do.	do.	Treated	16	7	43.8	Do.
	do.	do.	Trebl	Untreated	21	0	0	Do.
	do.	do.	do.	Treated	20	0	0	Do.
	do.	do.	Wisconsin Pedigree No. 5.	Untreated	33	16	48.5	<i>U. nuda</i> .
	do.	do.	do.	Treated	52	6	11.5	Do.
	do.	do.	Wisconsin Winter.	Untreated	85	25	29.4	Do.
	do.	do.	do.	Treated	167	23	13.8	Do.
	Wisconsin Pedigree No. 5.	do.	Alpha	Untreated	29	22	75.9	<i>U. nigra</i> .
	do.	do.	do.	Treated	41	0	0	Do.
	do.	do.	Comfort	Untreated	20	14	70.0	<i>U. nigra</i> .
	do.	do.	do.	Treated	32	0	0	Do.
	do.	do.	Colsess	Untreated	19	9	47.4	<i>U. nigra</i> .
	do.	do.	do.	Treated	31	0	0	Do.
101	do.	do.	Featherston	Untreated	53	0	0	Do.
	do.	do.	do.	Treated	73	0	0	Do.
	do.	do.	Glabron	Untreated	6	2	33.3	<i>U. nigra</i> .
	do.	do.	do.	Treated	8	0	0	Do.
	do.	do.	Hannchen	Untreated	13	11	84.6	<i>U. nigra</i> .
	do.	do.	do.	Treated	32	0	0	Do.
	do.	do.	Trebl	Untreated	40	22	55.0	<i>U. nigra</i> .
	do.	do.	do.	Treated	52	0	0	Do.
	do.	do.	Wisconsin No. 38.	Untreated	25	0	0	Do.
	do.	do.	do.	Treated	41	0	0	Do.
	do.	do.	Wisconsin Winter.	Untreated	74	41	55.4	<i>U. nigra</i> .
	do.	do.	do.	Treated	168	0	0	Do.
106	Esaw	do.	Alpha	Untreated	12	3	25.0	<i>U. nuda</i> .
	do.	do.	do.	Treated	85	4	11.4	Do.
	do.	do.	Wisconsin Winter.	Untreated	54	25	46.3	<i>U. nuda</i> .
107	do.	do.	do.	Treated	100	28	28.0	Do.
	Wisconsin Winter.	do.	do.	Untreated	69	36	52.2	<i>U. nigra</i> .
108	do.	do.	do.	Treated	146	0	0	Do.
	Beardless No. 6.	do.	Alpha	Untreated	28	2	7.1	<i>U. nuda</i> .
	do.	do.	do.	Treated	58	4	6.9	Do.
109	Alpha	do.	do.	Untreated	26	20	76.9	Both species.
	do.	do.	do.	Treated	28	8	28.6	<i>U. nuda</i> .
	do.	do.	Wisconsin Winter.	Untreated	80	41	51.3	Both species.
110	do.	do.	do.	Treated	168	6	3.6	<i>U. nuda</i> .
	Rowan	Raleigh, N. C.	Alpha	Untreated	10	9	90.0	<i>U. nigra</i> .
113	do.	do.	do.	Treated	12	0	0	Do.
	do.	Middleburg, Va.	do.	Untreated	13	12	92.3	Both species.
14	do.	do.	do.	Treated	18	2	11.1	<i>U. nuda</i> .
	do.	Hillsboro, Va.	do.	Untreated	13	12	92.3	Both species.
14	do.	do.	do.	Treated	20	1	5.0	<i>U. nuda</i> .

See footnote at end of table.

TABLE 4.—*Control of Ustilago nuda and U. nigra in plants of 11 varieties of barley grown from seed from flowers inoculated by hand with 1 or more of 22 collection of loose smut from various varieties and localities in the United States—Continued*

Smut collection no.	Variety and locality from which smut was collected in 1932		Varieties florally inoculated ¹	Seed (from inoculated flowers) untreated or treated with formaldehyde	Total number of plants	Smutted plants		Species of loose smut (<i>U. nuda</i> or <i>U. nigra</i>) collected from smutted heads in 1933
	Variety	Locality				Number	Percent	
115	Colsess	Rossllyn, Va.	Alpha	Untreated	18	6	33.3	Both species.
	do	do	do	Treated	27	6	22.2	<i>U. nuda</i> .
	do	do	Colsess	Untreated	20	7	35.0	Both species.
116	do	do	do	Treated	40	7	17.5	<i>U. nuda</i> .
	do	Watson, Va.	Alpha	Untreated	15	15	100.0	Both species.
	do	do	do	Treated	21	1	4.8	<i>U. nuda</i> .
117	Black Barblless	Rossllyn, Va.	do	Untreated	7	7	100.0	Both species.
	do	do	do	Treated	22	1	4.5	<i>U. nuda</i> .
	do	do	Comfort	Untreated	16	14	87.5	Both species.
119	do	do	do	Treated	30	1	3.3	<i>U. nuda</i> .
	do	do	Wisconsin Winter	Untreated	81	21	26.9	Both species.
	do	do	do	Treated	168	28	16.7	<i>U. nuda</i> .
120	Alpha	do	Alpha	Untreated	13	13	100.0	<i>U. nigra</i> .
	do	do	do	Treated	19	0	0	
	do	do	do	Untreated	42	42	100.0	<i>U. nigra</i> .
126	do	do	do	Treated	42	0	0	
	do	do	Comfort	Untreated	31	21	67.7	<i>U. nigra</i> .
	do	do	do	Treated	43	0	0	
129	do	do	Alpha	Untreated	37	16	43.2	<i>U. nuda</i> .
	do	do	do	Treated	37	15	40.5	Do.
	Spartan	do	do	Untreated	20	7	35.0	Both species.
133	do	do	do	Treated	31	9	29.0	<i>U. nuda</i> .
	do	do	Hannchen	Untreated	46	31	67.4	Both species.
	do	do	do	Treated	47	16	34.0	<i>U. nuda</i> .
134	do	Buchanan County, Mo.	Alpha	Untreated	13	8	61.5	<i>U. nigra</i> .
	do	do	do	Treated	19	0	0	
	do	do	Comfort	Untreated	12	11	91.7	<i>U. nigra</i> .
135	do	do	do	Treated	31	0	0	
	do	do	Wisconsin No. 38	Untreated	66	8	12.1	<i>U. nigra</i> .
	do	do	do	Treated	57	0	0	
136	do	Ithaca, N. Y.	Alpha	Untreated	6	2	33.3	<i>U. nuda</i> .
	do	do	do	Treated	18	4	22.2	Do.
	Alpha	do	do	Untreated	16	14	87.5	Both species.
137	do	do	do	Treated	25	7	28.0	<i>U. nuda</i> .
	do	do	Comfort	Untreated	34	20	58.8	Both species.
	do	do	do	Treated	45	4	8.9	<i>U. nuda</i> .
138	do	Marion, N. Y.	Alpha	Untreated	3	3	100.0	Both species.
	do	do	do	Treated	12	2	16.7	<i>U. nuda</i> .
	Wisconsin No. 38	Ithaca, N. Y.	do	Untreated	7	6	85.7	Both species.
139	do	do	do	Treated	18	5	27.8	<i>U. nuda</i> .
	do	do	Wisconsin No. 38	Untreated	12	7	58.3	Both species.
	do	do	do	Treated	21	6	28.6	<i>U. nuda</i> .

¹ Control plants also were grown, from seed of heads not artificially inoculated, of each of the varieties used in the floral inoculation test. 100 seeds of each variety were sown and from 83 to 100 plants matured. A trace of loose smut (*U. nigra*), resulting from natural infection, appeared in Wisconsin Winter. Control plants of the other varieties were smut-free.

The data in table 4 confirm and amplify those in tables 1 and 3 in showing that the treatment of seed from inoculated flowers with certain surface disinfectants is highly effective in the control of *Ustilago nigra* but relatively ineffective against *U. nuda*. In lots in which both species appeared in plants from untreated seed, *U. nigra* was completely controlled as a result of seed treatment and only *U. nuda* appeared in the plants from treated seed. Attention is directed particularly to the action of the formaldehyde seed treatment on control of the loose smuts in Wisconsin Winter in this experiment.

Naturally inoculated seed of this variety from the agronomy plots at the Arlington farm was used by Tisdale et al. (31, 32), Taylor and Zehner (27), and Leukel (20) in a series of seed-treatment studies, conducted in 9 of the 10 years from 1921 to 1930, in which treatment of the naturally inoculated seed with formaldehyde and various organic mercury solutions and several chemical dusts invariably resulted in effective control of the loose smut. In the writer's test (table 4) similar results were obtained when Wisconsin Winter was florally inoculated with the smut that naturally occurs on it at the Arlington farm (collection no. 107, table 4). Floral inoculations with *U. nigra* (collection no. 101) gave similar results. However, different results were obtained when the inoculations were made with collections that contained *U. nuda* alone (collections nos. 100 and 106) or in mixture with *U. nigra* (collections nos. 109 and 117). It is therefore evident from this and the other examples in table 4 that treatment of barley seed from inoculated flowers with surface disinfectants may eradicate loose smut, partially control it, or prove ineffective in control, depending upon the loose smut or smuts carried by the seed and irrespective of the barley variety concerned. Doubtless to this fact may be attributed the conflicting reports cited under Review of Previous Investigations or regarding the control of loose smut in barley through treatment of the seed with surface disinfectants.

Of the varieties florally inoculated with *Ustilago nuda*, seed treatment effected partial control in some cases, as shown in table 4. This may have been because some of the florets were inoculated after the fertilized ovaries had begun to enlarge. The very rapid rate of development of barley kernels following fertilization has been shown by Harlan (6). Apparently the depth of penetration of the infection hyphae into the young seed tissues is restricted under these conditions and the hyphae are consequently less protected from the action of surface or subsurface disinfectants applied to the seed. As noted in table 1, partial control of *U. nuda* (collection no. 100) also was obtained by inoculating flowers of Featherston and Wisconsin Pedigree No. 5 when the ovaries were one-fourth to one-third developed and the seed was treated with Ceresan dust. Freeman and Johnson (5) have reported similar results.

With reference to varietal resistance and susceptibility, table 4 shows that Featherston was resistant to *Ustilago nuda* (collection no. 100) and immune to *U. nigra* (collection no. 101). In earlier experiments, however, this variety proved fairly susceptible to *U. nuda* (collection no. 100, table 1) when grown from seed from inoculated flowers, and only moderately resistant to *U. nigra* (collection no. 101, table 2) when grown from seed inoculated at the time of sowing. In these experiments, therefore, seed inoculation with *U. nigra* proved more effective than floral inoculation in the production of smutted plants in Featherston. Trebi proved immune to *U. nuda* (collection no. 100) but highly susceptible to *U. nigra* (collection no. 101). None of the other varieties was markedly resistant to either of the loose smuts. Alpha and Hannchen were highly susceptible to both *U. nuda* and *U. nigra*.

The foregoing comparative studies of *Ustilago nuda* and *U. nigra* relative to the production of smutted plants as a result of seed inoculation and seedling infection have been limited to three smut collections (nos. 100, 101, and 106), as shown in table 2. The results of

further seed-inoculation studies, made in the spring of 1933 at the Arlington farm, are presented in table 5. Five loose smut collections (nos. 110, 120, 126, 133, and 134) not used in the previous seed-inoculation test were employed. They were collected from plants grown in a greenhouse and used in the preceding experiment. The seed of the varieties inoculated was first treated with hot water (modified method), left to dry during several days, blackened with smut, and then immediately sown in deep flats in moderately moist soil maintained close to 21° C. until the seedlings had fully emerged. Then the plants in flats were placed outdoors to complete growth. A germination test of each of the smut collections at sowing time showed that all were highly viable. Results from a seed inoculation of Esaw with smut collection no. 101 (*U. nigra*) also are included in table 5 because they show an influence of the host from which the smut is collected on the pathogenicity of the inoculum under certain conditions.

TABLE 5.—Incidence of loose smut in varieties of barley as a result of inoculating mature seed with 2 collections of *Ustilago nuda* and 4 of *U. nigra*

Collection no of inoculum	<i>Ustilago</i> species of loose smut	Variety from which smut was collected in—		Variety inoculated (inoculum applied to mature seed) ¹	Total number of plants	Smutted plants	
		1932	1933			Number	Percent
101.....	<i>U. nigra</i>	Alpha.....	Esaw.....	Esaw.....	93	36	38.7
		do.....	Trebi.....	do.....	88	4	4.5
		Rowan.....	Alpha.....	Alpha.....	38	4	10.5
110.....	do.....	do.....	do.....	Hannchen.....	46	0	.0
		do.....	do.....	Trebi.....	42	0	.0
120.....	do.....	Alpha.....	do.....	Alpha.....	36	14	38.9
		do.....	do.....	Hannchen.....	42	12	28.6
		do.....	do.....	Trebi.....	42	13	31.0
133.....	do.....	do.....	do.....	Alpha.....	30	4	13.3
		do.....	do.....	Hannchen.....	46	10	21.7
		do.....	do.....	Trebi.....	28	2	7.1
126.....	<i>U. nuda</i>	Alpha.....	do.....	Alpha.....	70	0	.0
		do.....	do.....	Hannchen.....	91	0	.0
		do.....	do.....	Trebi.....	86	0	.0
134.....	do.....	do.....	do.....	Alpha.....	67	0	.0
		do.....	do.....	Hannchen.....	92	0	.0
		do.....	do.....	Trebi.....	83	0	.0

¹ From 78 to 95 control plants from uninoculated seed of each of the varieties also were grown, and were smut-free.

The data of table 5 show that all of the *Ustilago nigra* collections (nos. 101, 110, 120, and 133) were able to produce smutted plants as a result of seed inoculation and seedling infection. The *U. nuda* collections (nos. 126 and 134) were wholly unable, however, to produce smutted plants through seed inoculation despite the fact that, as previously shown (table 4), at least in the variety Alpha, they were able to produce high percentages of smut in plants grown from seed from inoculated flowers. These results confirm those obtained in the previous seed-inoculation test (table 2) with *U. nuda* (collections nos. 100 and 106) and *U. nigra* (collection no. 101).

The data of table 5 also show that when Esaw was seed-inoculated with smut collection 101 (*Ustilago nigra*) from Esaw a considerably higher percentage of infected plants was obtained than when it was inoculated with smut collection 101 (*U. nigra*) from Trebi. The Esaw and Trebi plants that furnished the smut in 1933 were grown from seed from flowers inoculated with the same smut collection

(*U. nigra* no. 101) from Alpha in 1932. Tisdale and Griffiths obtained similar results with other barleys and loose smut collections and suggested that the host variety might affect the pathogenicity of the inoculum through a definite influence on either (1) the parasitism of a single physiologic form or (2) alteration in the relative content of two or more component physiologic forms.

USTILAGO NIGRA IN RELATION TO PREVIOUS INVESTIGATIONS

Knowledge of the occurrence of *Ustilago nigra* in the United States appears to explain the conflicting reports relative to barley-seed treatment with surface disinfectants as previously noted, and also seems to facilitate an interpretation of the hitherto unexplained results of the previous investigations of Tisdale and Tapke (30) and Taylor and Zehner (28).

In 1924 Tisdale and Tapke (30), unaware of the existence of *Ustilago nigra*, reported their discovery of the "infection of barley by *Ustilago nuda* through seed inoculation." In the present investigation, however, as shown in tables 2 and 5, the results of the writer have confirmed those of previous investigators (2, 3, 5, 10, 16) in showing that *U. nuda* is wholly incapable of producing smutted plants as a result of the inoculation of mature barley seed with smut spores. That the loose smut used by Tisdale and Tapke (30) was *U. nigra* seems likely in view of the fact that their inoculum was collected from Tennessee Winter grown at the Arlington farm in 1922. Examination by the writer of collections of the loose smut naturally occurring on this variety at that place for the past 5 years has shown that only *U. nigra* was present. Furthermore, in a series of seed-treatment studies conducted by Tisdale et al. (31, 32), Taylor and Zehner (27), and Leukel (20), in 9 of the 10 years from 1921 to 1930, naturally inoculated seed of Tennessee Winter from the Arlington farm was among the varieties employed, and its loose smut invariably proved amenable to control through treatment of the naturally inoculated seed with surface disinfectants. As shown in tables 1, 3, and 4, *U. nuda* is not controlled by this measure.

That Tisdale and Tapke (30) were working with *Ustilago nuda* and not *U. nigra* might be assumed from the fact that in a cytologic examination of seedlings grown from inoculated seed on moist paper, at approximately 21° C., it was clearly shown that the promycelia of the germinating chlamydospores directly penetrated the tissue of the coleoptile and young leaves. In no instance did they bear the sporidia characteristic of *U. nigra* when germinated on potato-dextrose agar. The production or nonproduction of sporidia by the promycelium of the germinating chlamydospore cannot be used, however, as a diagnostic character of the species under all conditions. Promycelia of *U. nigra* may be made to produce sporidia abundantly or sparsely or to branch freely without production of any sporidia by using different substrata on which to germinate the spores. Jones (14) and Hüttig (11) have obtained similar results with other cereal-smut fungi through the use of low or high temperatures, and Kolk (17) also has observed the direct penetration of an oat seedling by the germ tube of a chlamydospore of the loose smut fungus of oats (*U. avenae* (Pers.) Jens.), which normally produces sporidia in culture.

In 1931 Taylor and Zehner (28) reported their results on the effect of depth of seeding on the occurrence of covered and loose smuts in

winter barley at the Arlington farm. They found that both loose and covered smuts were materially increased in the Wisconsin Winter variety when the naturally inoculated seed was sown 3 inches deep, as compared with their occurrence when the seed was sown one-half inch deep. However, the varieties Esaw and Beardless No. 6, also grown from naturally inoculated seed, "showed no significant difference in loose-smut infection from variation in seeding depth." Owing to resistance, the two last-named varieties yielded no data on covered smut. In 1932, the writer collected loose smut at the Arlington farm from each of the same three barleys previously used by Taylor and Zehner. These smuts were used in 1932 and 1933 in the floral-inoculation seed-treatment experiment summarized in table 4. Conclusive evidence was obtained that the smut collected from Wisconsin Winter was *Ustilago nigra* and the smuts from Esaw and Beardless No. 6 were *U. nuda*. Further studies on the color of the sifted spore masses of these smuts and their mode of germination on 2-percent potato-dextrose agar confirmed their identity as noted. It seems likely, therefore, that Taylor and Zehner also were dealing with *U. nigra* on Wisconsin Winter and *U. nuda* on Esaw and Beardless No. 6. On this assumption the results of Taylor and Zehner might be explained as follows: Hecke (9), Broilli (3), Broilli and Schickorra (4), and Lang (18) have shown that following floral inoculation *U. nuda* penetrates deeply within the developing seed and has thoroughly ramified the tissues of the embryo and endosperm by the time the seed is ripe. Therefore, infection, the critical point in the life of the parasite, has been well established before sowing, and subsequent influences brought about by variation in depth of seeding would not apply to this phase. In the case of *U. nigra* on Wisconsin Winter, however, the growth of the fungus following floral infection appears to be confined to the superficial layers of the seed, as evidenced by the fact that surface disinfection of the seed with formaldehyde solution effects complete control (table 4). With *U. nigra*, therefore, infection of the seedling remains to be accomplished when the seed is ripe, and variation in environment or in seedling susceptibility brought about by different depths of seeding might reasonably be expected to exert an influence on infection and on the degree of smuttedness in the plants.

CONCLUSIONS

Knowledge of the occurrence of *Ustilago nigra* in the United States seems to explain the variable results in control hitherto obtained from the application of surface disinfectants to barley seed harboring loose smut. It should also effect a reduction in loss from loose smut through a wider use of the easily applied surface disinfectants where it is known that the *nigra* species is the causal organism. Knowledge of the occurrence of *U. nigra* doubtless also should facilitate studies on (1) the genetic relation of the barley smuts, (2) the occurrence of physiologic forms in the barley loose smuts, and (3) breeding for resistance to these smuts.

SUMMARY

During the past 20 years it has been frequently reported in the United States that loose smut in barley may be reduced or eliminated through treatment of the harboring seed with certain easily applied surface disinfectants. This is at variance with the reports of all of

the earlier and some of the recent investigators, who have found that it is necessary to apply the difficult modified hot-water treatment or its equivalent in order to control loose smut in barley through seed treatment.

The present investigation has shown that in addition to the well-known loose smut of barley caused by *Ustilago nuda* (Jens.) Kell. and Sw. a second barley loose smut caused by *U. nigra* Tapke is widely distributed and causes considerable loss in the United States. The latter species is controllable through treatment of the seed with certain surface disinfectants, and doubtless has been the factor responsible for the conflicting reports noted above.

Ustilago nigra resembles *U. nuda* in the appearance of smutted heads, in the emergence of smutted heads and dissemination of spores during the heading and flowering period of unsmutted plants, and in the inoculation of the host through the flowers.

Ustilago nigra may be distinguished from *U. nuda* by the following characteristics: (1) The color of the spore mass of *U. nigra* on fully emerged heads is dark chocolate-brown, whereas that of *U. nuda* is olivaceous brown. (2) In seed from flowers inoculated at blooming, *U. nigra* is amenable to control through seed treatment with certain surface disinfectants. The modified hot-water treatment or its equivalent is necessary to control *U. nuda*. (3) On 2-percent potato-dextrose agar at 70° F., the spores of *U. nigra* germinate by producing a promycelium bearing typically four lateral sporidia. Under these conditions the spores of *U. nuda* produce a promycelium that does not bear sporidia. (4) *U. nigra* is able to produce smutted plants through infection of the seedling as a result of inoculating mature barley seed, as well as through infection of the flowers. *U. nuda* is able to produce infection only through the flowers.

Some of the most important varieties of barley in the United States are highly susceptible to *Ustilago nigra*.

To facilitate distinction, the terms "brown" and "black" loose smut of barley are proposed for *Ustilago nuda* and *U. nigra*, respectively.

LITERATURE CITED

- (1) BREFELD, O.
1903. NEUE UNTERSUCHUNGEN UND ERGEBNISSE ÜBER DIE NATÜRLICHE INFEKTION UND VERBREITUNG DER BRANDKRANKHEITEN DES GETREIDES. Nachr. Klub Landw. Berlin 466: 4224-4234.
- (2) ——— and FALCK, R.
1905. DIE BLÜTENINFEKTION BEI DEN BRANDPILZEN UND DIE NATÜRLICHE VERBREITUNG DER BRANDKRANKHEITEN. In Brefeld, O., Untersuchungen aus dem Gesamtgebiete der Mykologie, Heft 13, 74 pp., illus. Münster.
- (3) BROILI, J.
1910. VERSUCHE MIT BRAND-INFEKTION ZUR ERZIELUNG BRANDFREIER GERSTENSTÄMME. Naturw. Ztschr. Forst u. Landw. 8: 335-344, illus.
- (4) ——— and SCHIKKORRA, W.
1913. BEITRÄGE ZUR BIOLOGIE DES GERSTENFLUGBRANDES (USTILAGO HORDEI NUDA JEN.). Ber. Deut. Bot. Gesell. 31: 336-339, illus.
- (5) FREEMAN, E. M., and JOHNSON, E. C.
1909. THE LOOSE SMUTS OF BARLEY AND WHEAT. U. S. Dept. Agr., Bur. Plant Indus. Bull. 152, 48 pp., illus.
- (6) HARLAN, H. V.
1920. DAILY DEVELOPMENT OF KERNELS OF HANNCHEN BARLEY FROM FLOWERING TO MATURITY AT ABERDEEN, IDAHO. Jour. Agr. Research 19: 393-430, illus.

- (7) HARLAN, H. V., and ANTHONY, S.
1920. DEVELOPMENT OF BARLEY KERNELS IN NORMAL AND CLIPPED SPIKES AND THE LIMITATIONS OF AWNLESS AND HOODED VARIETIES. *Jour. Agr. Research* 19: 431-472, illus.
- (8) HECKE, L.
1904. EIN INNERER KRANKHEITSKEIM DES FLUGBRANDES IM GETREIDE-KORN. *Ztschr. Landw. Versuchsw. Österr.* 7: 59-64.
- (9) ———
1905. ZUR THEORIE DER BLÜTENINFEKTION DES GETREIDES DURCH FLUGBRAND. *Ber. Deut. Bot. Gesell.* 23: 248-250, illus.
- (10) HORI, S.
1907. SEED INFECTION BY SMUT FUNGI OF CEREALS. *Bull. Imp. Cent. Agr. Expt. Sta. Japan* 1: 163-176.
- (11) HÜTTIG, W.
1931. ÜBER DEN EINFLUSS DER TEMPERATUR AUF DIE KEIMUNG UND GESCHLECHTER VERTEILUNG BEI BRANDPILZEN. *Ztschr. Bot.* 24: [529]-577, illus.
- (12) JENSEN, J. L.
1888. THE PROPAGATION AND PREVENTION OF SMUT IN OATS AND BARLEY. *Jour. Roy. Agr. Soc. England* (2) 24: 397-415.
- (13) JOHNSON, A. G.
1914. EXPERIMENTS ON THE CONTROL OF CERTAIN BARLEY DISEASES. (Abstract) *Phytopathology* 4: 46.
- (14) JONES, E. S.
1923. INFLUENCE OF TEMPERATURE, MOISTURE, AND OXYGEN ON SPORE GERMINATION OF *USTILAGO AVENAE*. *Jour. Agr. Research* 24: 577-591, illus.
- (15) KELLERMAN, W. A., and SWINGLE, W. T.
1890. REPORT ON THE LOOSE SMUTS OF CEREALS. *Kans. Agr. Expt. Sta. Ann. Rept.* (1889) 2: 213-288, illus.
- (16) KIRBY, R. S.
1927. DISEASES OF SMALL GRAINS. *N. Y. Agr. Col. (Cornell) Ext. Bull.* 157, 71 pp., illus.
- (17) KOLK, L. A.
1930. RELATION OF HOST AND PATHOGEN IN THE OAT SMUT, *USTILAGO AVENAE*. *Bull. Torrey Bot. Club* 57: 443-507, illus.
- (18) LANG, W.
1917. ZUR ANSTECKUNG DER GERSTE DURCH *USTILAGO NUDA*. *Ber. Deut. Bot. Gesell.* 35: 4-20.
- (19) LEIGHTY, C. E., and SANDO, W. J.
1925. A HANDY POLLEN CARRIER. *Jour. Heredity* 16: 63-65, illus.
- (20) LEUKEL, R. W.
1932. FACTORS AFFECTING THE DEVELOPMENT OF LOOSE SMUT IN BARLEY AND ITS CONTROL BY DUST FUNGICIDES. *U. S. Dept. Agr. Tech. Bull.* 293, 20 pp.
- (21) MADDOX, F.
1895. EXPERIMENTS AT EASTFIELD. SMUT, BUNT, RUST. *Tasmania Dept. Agr.* 4 pp.
- (22) REDDY, C. S., and BURNETT, L. C.
1930. DEVELOPMENT OF SEED TREATMENTS FOR THE CONTROL OF BARLEY STRIPE. *Phytopathology* 20: 367-390, illus.
- (23) RUTTLE, M. L. (MRS. NEBEL).
1934. STUDIES ON BARLEY SMUTS AND ON LOOSE SMUT OF WHEAT. *N. Y. Agr. Expt. Sta. Tech. Bull.* 221, 39 pp., illus.
- (24) TAPKE, V. F.
1929. INFLUENCE OF VARIETAL RESISTANCE, SAP ACIDITY, AND CERTAIN ENVIRONMENTAL FACTORS ON THE OCCURRENCE OF LOOSE SMUT IN WHEAT. *Jour. Agr. Research* 39: 313-339, illus.
- (25) ———
1931. INFLUENCE OF HUMIDITY ON FLORAL INFECTION OF WHEAT AND BARLEY BY LOOSE SMUT. *Jour. Agr. Research* 43: 503-516, illus.
- (26) ———
1932. AN UNDESCRIBED LOOSE SMUT OF BARLEY. (Phytopathological Note) *Phytopathology* 22: 869-870.

- (27) TAYLOR, J. W., and ZEHNER, M. G.
1930. THE EFFECT OF A SEED DISINFECTANT ON GRAIN AND STRAW YIELDS AND SMUT CONTROL IN WINTER BARLEY. *Jour. Amer. Soc. Agron.* 22: 113-123, illus.
- (28) ——— and ZEHNER, M. G.
1931. EFFECT OF DEPTH OF SEEDING ON THE OCCURRENCE OF COVERED AND LOOSE SMUTS IN WINTER BARLEY. *Jour. Amer. Soc. Agron.* 23: 132-141, illus.
- (29) TISDALE, W. H., and GRIFFITHS, M. A.
1927. VARIANTS IN *USTILAGO NUDA* AND CERTAIN HOST RELATIONSHIPS. *Jour. Agr. Research* 34: 993-1000.
- (30) ——— and TAPKE, V. F.
1924. INFECTION OF BARLEY BY *USTILAGO NUDA* THROUGH SEED INOCULATION. *Jour. Agr. Research* 29: 263-284, illus.
- (31) ——— TAYLOR, J. W., and GRIFFITHS, M. A.
1923. EXPERIMENTS WITH HOT WATER, FORMALDEHYDE, COPPER CARBONATE, AND CHLOROPHOL FOR THE CONTROL OF BARLEY SMUTS. *Phytopathology* 13: 153-160.
- (32) ——— TAYLOR, J. W., LEUKEL, R. W., and GRIFFITHS, M. A.
1925. NEW SEED DISINFECTANTS FOR THE CONTROL OF BUNT OF WHEAT AND THE SMUTS OF OATS AND BARLEY. *Phytopathology* 15: [651]-676, illus.
- (33) ZEINER, W.
1932. DAS VERHALTEN VERSCHIEDENER SOMMERGERSTEN-KREUZUNGEN HINSICHTLICH DER ANFÄLLIGKEIT FÜR *USTILAGO NUDA*. *Ztschr. Züchtung, A, Pflanzenzücht.* 17: [229]-264, illus.

THE SUGAR BEET NEMATODE AND OTHER INDIGENOUS NEMIC PARASITES OF SHADSCALE¹

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INTRODUCTION

The original sources of nemic parasites are generally unknown. It is, therefore, of considerable interest to find the sugar beet nematode (*Heterodera schachtii* Schmidt, 1871), *Anquillulina aberrans* n. sp., and *Neotylechus latus* n. sp. under conditions that apparently leave no doubt as to their native origin. On May 25, 1927, *H. schachtii* and *A. aberrans* were found infesting the roots of shadscale, *Atriplex confertifolia* (Torr. and Frem.) S. Wats., collected in the desert foothills west of Utah Lake, Utah. Collections made in 1933 extended the desert range of these two nemas into Cedar Valley, several miles west of the first collection, and to the hills west of Richfield, about 95 miles south. *N. latius* also was found in the material collected west of Richfield and in a single collection made west of Utah Lake.

THE SUGAR BEET NEMATODE

The fact that the sugar beet nematode (*Heterodera schachtii*) is indigenous to the desert is not surprising when one considers the morphology of the brown cyst stage. This remarkable adaptation to adverse circumstances doubtless originated under ecological conditions similar to those of its present native habitat. That this species is indigenous to both Europe and North America is not surprising, for many nemas are cosmopolitan. The Division of Nematology collection at Salt Lake City, Utah, contains over 100 species which are native to virgin soils of the United States and which also have been reported from Europe and other parts of the world.

Previous reports have indicated that *Heterodera schachtii* is a species indigenous to the United States. Maxson² found specimens on *Rumex* sp. in the Salinas Valley, Calif., in 1913, which he believed could not have come from introduced nemas. Steiner³ discovered *Polygonum pensylvanicum* L. and *P. punctatum* Ell. infested with *H. schachtii* at Broad Run, near Leesburg, Va., under circumstances that might indicate native colonies in that locality.

These indigenous colonies of sugar beet nematodes explain the probable source of many infestations in the beet fields of Utah. Originally a considerable portion of the present beet land was "shadscale flats", comparatively level areas bearing almost pure stands of shadscale which were destroyed by cultivation. There is a possibility that the nemas lived on some desert plants that survived

¹ Received for publication June 26, 1935; issued December 1935.

² Oral communication in 1934 from A. C. Maxson, Great Western Sugar Co., Denver, Colo.

³ STEINER, G. THE FINDING OF *HETERODERA SCHACHTII*, THE SUGAR BEET NEMA, ON *POLYGONUM* IN VIRGINIA. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr. 15: 145. 1931. [Mimeographed.]

cultivation, or perhaps they transferred from shadscale to new hosts, such as species of *Brassica*, *Rumex*, and *Atriplex*, on which they lived until sugar beets were introduced many years later.

Native colonies doubtless do not account for all nemic infestations now present in beet-growing areas. Transmission of nemas in beet seed from Europe has been known to occur⁴ and probably is responsible for many infestations.

Comparisons of specimens from the desert with those from beet fields show only slight variations in form and size, the principal difference being in the length of the male tails (fig. 1, *B*), which in the

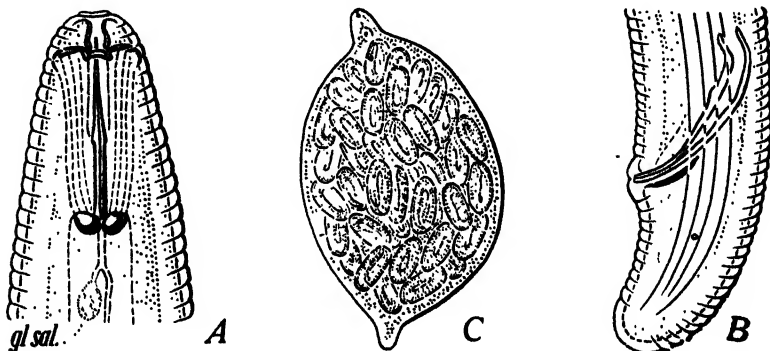


FIGURE 1.—*Heterodera schachtii*: A, Male head; *gl. sal.*, salivary gland, $\times 1,300$. B, Male tail, $\times 1,000$. C, Cyst, $\times 60$.

desert form are 2 to 3 times as long as those of nemas found in beet fields. Long-tailed specimens occasionally are found living on beets in Utah, and Cobb⁵ figures a similar form from Colorado.

EFFECT ON HOST

Infestations generally are limited to small groups of shadscale bushes, and there may be several rods, or even a mile, between infestations. However, the locating of infested plants is largely a matter of chance, for no visible injury or appreciable damage is shown by *Brassica*, *Atriplex*, and other common weed hosts. Infested bushes frequently are found along a watercourse and about its drainage basin.

ANGUILLULINA ABERRANS N. SP.

Anguillulina aberrans, n. sp., exhibits characters that indicate a relationship to both *Heterodera* and *Tylenchulus*. The male head of *A. aberrans* (fig. 2, *C*) is indistinguishable from that of *H. schachtii* (fig. 1, *A*). The distorted, variable-formed females of *A. aberrans* are suggestive of the females of the root knot nematode, *H. marioni* (Cornu, 1879) Goodey, 1932, from hard, woody tissues in which the body is unable to assume its typical pyriform shape. The chief difference lies in the appearance of the female terminus, which retains its identity in *A. aberrans* but completely loses it in *H. marioni*.

⁴ THORNE, G. CONTROL OF THE SUGAR-BEET NEMATODE BY CROP ROTATION. U. S. Dept. Agr. Farmers' Bull. 1514, 21 pp., illus. 1926. See p. 6.

⁵ COBB, N. A. ESTIMATING THE NEMA POPULATION OF SOIL, WITH SPECIAL REFERENCE TO THE SUGAR-BEET AND ROOT-GALL NEMAS, *HETERODERA SCHACHTII* SCHMIDT AND *HETERODERA RADICICOLA* (GREEF) MÜLLER, AND WITH A DESCRIPTION OF *TYLENCHOLAIMUS AEQUALIS* N. SP. U. S. Dept. Agr., Bur. Plant Indus. Agr. Technol. Circ. 1, 46 pp., illus. 1918. See p. 25, fig. 17.

The females of *Anguillulina aberrans* are in many respects similar in development and appearance to the females of *Tylenchulus semipenetrans* Cobb⁶, but the former species differs from *Tylenchulus* in possessing the following characters: Anus functional, excretory pore near base of esophagus, male spear normal, and bursa present.

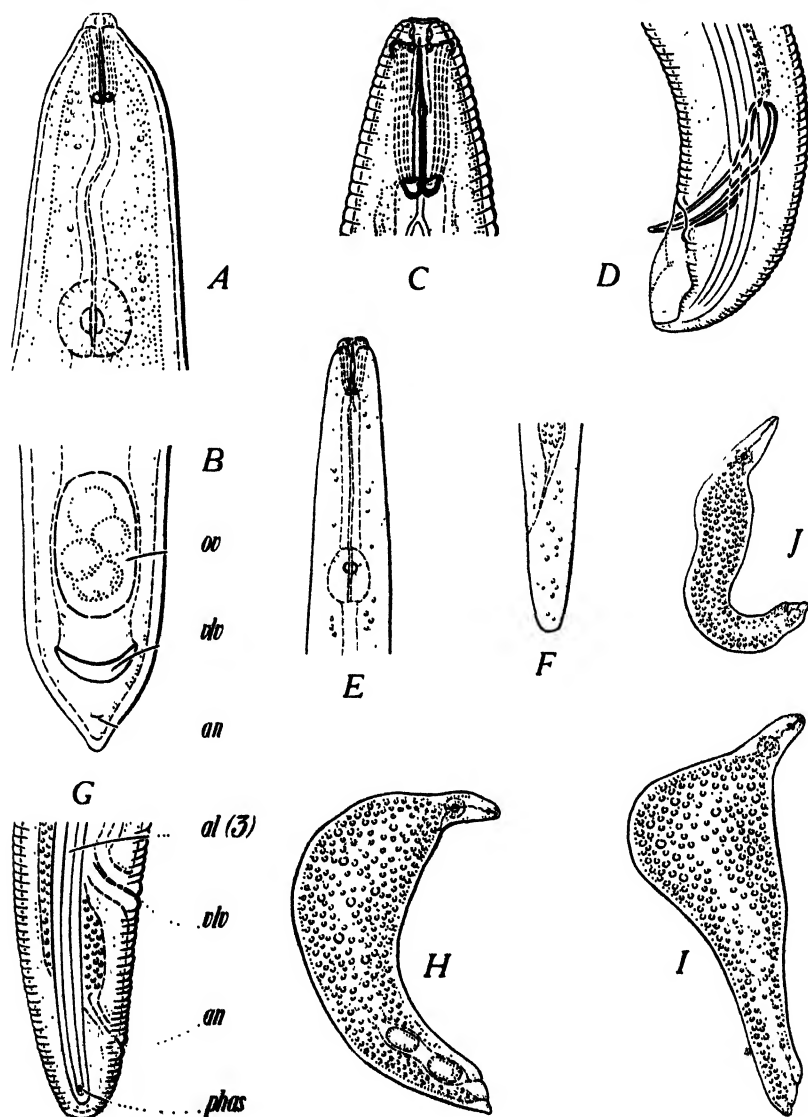


FIGURE 2.—*Anguillulina aberrans*. A, Anterior end of adult female, $\times 500$. B, Posterior end of adult female: *ov*, segmenting egg; *seg*, vulva; *an*, anus, $\times 250$. C, Male head, $\times 1,000$. D, Posterior portion of male, $\times 1,000$. E, Anterior end of larva, $\times 500$. F, Posterior end of larva, $\times 500$. G, Posterior end of young female: *al* (3), wings (three); *vb*, vulva; *an*, anus; *phas*, phasmid, $\times 500$. H, I, J, Variations in size and form of adult females, $\times 60$.

⁶ COBB, N. A. NOTES ON MONONCHUS AND TYLENCHULUS. Jour. Wash. Acad. Sci. 3: 287-288, illus. 1913.

MORPHOLOGY

Eggs.—Average size $42\mu \times 80\mu$. Found within the roots. Deposited before segmentation in masses covered by gelatinous secretion from the female. Senile females frequently contain a few undeposited eggs in which segmentation and hatching may occur within the body (fig. 2, B).

Larvae (fig. 2, E, F).—Length 0.4 to 0.48 mm. Width 20μ to 24μ . Spear length 14μ . Esophagus occupying about one-fifth body length. Intestine densely granular, granules varying in size from very minute to one-fourth body width. Tail length generally about twice anal body diameter, tapering but little to broad, rounded terminus.

Male (fig. 2, C, D).—Length 0.7 to 0.9 mm. Width 25μ to 33μ . Body almost cylindrical between esophagus and anus, tapering to lip region, which is not set off in any manner. Tail slightly longer than anal body diameter, completely enveloped by bursa. Wings three, marked by four refractive lines, the area occupying one-fourth of body width. Six obscure, low, flat lips grouped close about the entrance to vestibule; from lateral view they frequently give appearance of very thin disk. Cuticular framework of lips massive, six-parted. Spear 21μ to 25μ long with large basal knobs. Median esophageal bulb about half as wide as neck. Terminal bulb obscure, assimilated by intestine. Excretory pore distinct, located 1 or 2 body widths behind median bulb. Testis single, outstretched. Spicula slightly arcuate, tapering in distal two-thirds. Gubernaculum thin, flat, slightly arcuate. Bursa rising close in front of anus, enveloping the ventrally arcuate tail. Phasmid riblike, near middle of bursa.

Young female (fig. 2, G).—Length 0.7 to 0.9 mm. Width 30μ to 35μ . Vulva at 91 to 93 percent. Body practically cylindrical from esophagus to vulva. Tail convex-conoid to broad, rounded terminus, its length equal to anal body diameter. Cuticle coarsely annulated. Wings three, marked by four refractive lines, the areas occupying about one-fourth of body width. Ovary immature. Posterior uterine branch absent.

Adult female.—Length 0.8 to 1.2 mm. Width 132μ to 345μ . Spear length 20μ to 24μ . Body distorted, variable in form (fig. 2, H, I, J), neck and posterior end retaining a slight semblance of original form. Median bulb almost spherical with strong radial muscles. Terminal bulb merging with intestines. Body densely granular. Details of the coiled single ovary completely obscured. Vulva broad transverse slit. Anus inconspicuous. Found only in root tissues.

Diagnosis.—*Anguillulina*. Spear massive, strongly knobbed. Labial framework strong, cuticularized. Adult female swollen, distorted, variable in size and form, found only in roots of host. Young female of normal anguilluloid form. Vulva at 91 to 93 percent. Ovary single. Posterior uterine branch absent. Tail rounded, about as long as anal body diameter. Phasmids near terminus. Male tail about as long as anal body diameter, ventrally bent, enveloped by bursa. Phasmid riblike, inserted in bursa.

Host: *Atriplex confertifolia*.

LIFE HISTORY

Specimens collected December 12, 1933, were preadults with an occasional larva. The preadults pass through summer drought and winter cold, becoming active in April, when the soil is moist and warm and when shadscale growth begins. Differentiation of sexes occurs at the last molt, the males and young females being present both within the roots and in the surrounding soil. Because of the difficulty males would experience in locating females within the roots, it is probable that copulation occurs in the soil, after which the young females enter the roots. The female lengthens but little as it becomes a distorted, misshapen mass, forcing its way into the hard root tissues; the body pressure becomes so great that the nemas usually burst when the root is opened. Eggs are deposited in masses and covered with a gelatinous secretion. Segmentation and hatching occur immediately and the larvae develop into preadults, which become quiescent until revived in spring.

EFFECT ON HOST

Roots of infested bushes appear very much as if they were infested with *Heterodera marioni*, irregular swellings and occasionally typical galls being formed. As a result of infestation, many bushes are killed or severely injured. This injury is especially noticeable in years that follow a dry season. Such a condition was very evident

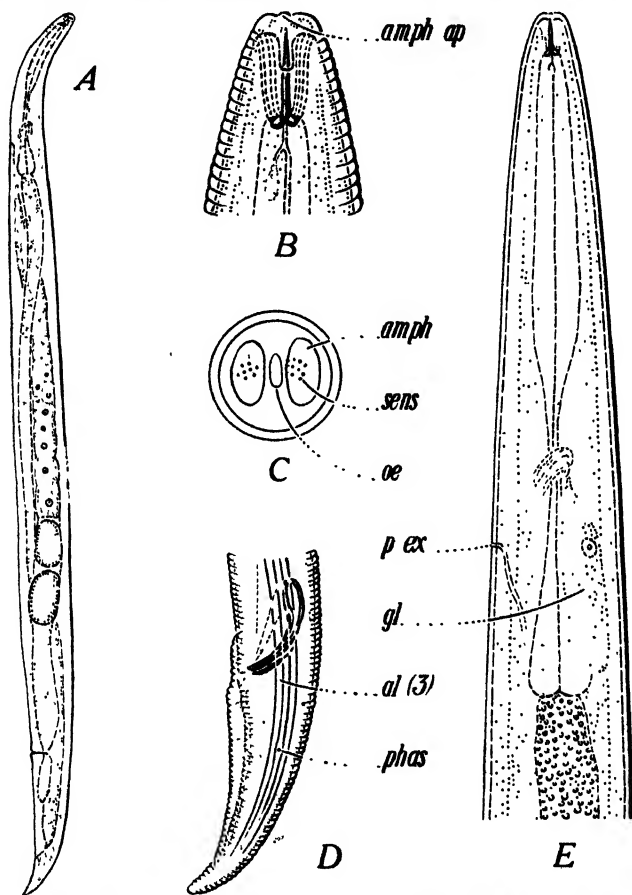


FIGURE 3.—*Neotylelenchus latus*: A, Adult female, $\times 120$. B, Head: *amph ap*, amphid aperture, $\times 1,500$. C, Cross section of neck just posterior to spear: *amph*, amphid; *sens*, sensillae; *oe*, esophagus, $\times 1,500$. D, Male tail: *al* (3), wings (three); *phas*, phasmid, $\times 500$. E, Anterior end of female: *p ex*, excretory pore; *gl*, gland, $\times 500$.

in 1933 after the dry year of 1931. In 1931 the precipitation was only 11.4 inches and the mean for the 5-year period 1928–32 was 13.71 inches, as compared with a general mean of 16.18 inches.

NEOTYLELENCHUS LATUS N. SP.

Eight females of *Neotylelenchus latus* (fig. 3) were secured from the cortex of shadscale roots near Richfield, Utah. Two males and four females were found under similar conditions in a root taken from a spot west of Utah Lake. The species appears to be a rare inhabitant of shadscale roots, too few in number to be of any significance as a parasite.

MORPHOLOGY

Measurements:

1.	11.	16.	W	⁸⁸ 85. ⁶	94.5	0.7-1.1 mm	
1.	3.8	4.	5.9	5.1	2.		
1.	15.	21.	⁸⁸ M	95.	0.8 mm		
1.	2.2	2.4	3.2	2.			

Female.—Body attaining its greatest width at about 65 percent. Anteriorly tapers uniformly to lip region, which is not set off in any manner (fig. 3, *E*). Tail conoid to pointed terminus, its length about two and one-half times anal body diameter (fig. 3, *A*). Cuticle finely annulated. Wings three, marked by four refractive lines. Face view of lip region shows the 8 lip sections and 4 papillae characteristic of the genus.⁷ Amphid apertures at apex of lateral lips (fig. 3, *B*). Amphidial pouch half as wide as head, located nearly opposite base of spear, practically invisible from lateral view. Sensilla elements apparently eight. Spear 10 μ to 12 μ long, with three small basal knobs. Esophagus: Corpus comprising half the total length; isthmus exceedingly slender; bulb variable, generally elongate-conoid, half as wide as neck. Large gland close to bulb (fig. 3, *E*). Excretory pore just behind nerve ring. Intestine densely granulated, generally crowded to one side by ovary. Female prodelphic, ovary outstretched, reaching almost to base of esophagus. Posterior uterine branch reaching from one-third to three-fourths the distance to anus. Eggs about as long as greatest body diameter and half as wide as long. Vulva broad, transverse slit. Anus indistinct. Phasmids slightly anterior to middle of tail.

Male.—Body much less robust than that of female. Spear normal. Testis single, outstretched. Spicula arcuate, tapering. Gubernaculum thin, flat, slightly arcuate. Bursa crenate, striated, rising about opposite middle of spicula and extending to near terminus. Tail conoid, ventrally bent, ending in small rounded terminus (fig. 3, *D*).

Diagnosis.—*Neotylenchus* with above measurements. Differs from *N. abulbosus* Steiner, 1931, in the following characters: Tail shorter; vulva located farther in front of anus; posterior uterine branch present; male with spear, spicula, and gubernaculum, normal, not undeveloped as in *N. abulbosus*.⁸ *N. obesus* Thorne, 1934, is a much more obese species, with vulva located at 95 percent to almost terminal.

Host: *Atriplex confertifolia*.

SUMMARY

The sugar beet nematode, *Heterodera schachtii* Schmidt, 1871, is reported as an indigenous parasite of *Atriplex confertifolia* (Torr. and Frem.) S. Wats. in Utah. *Anguillulina aberrans* n. sp., and *Neotylenchus latus* n. sp., are also discussed as parasites of *A. confertifolia*, and morphological diagnoses are given.

⁷ STEINER, G. NEOTYLENCHUS ABULBOSUS N. G., N. SP. (TYLENCHIDAE, NEMATODA) THE CAUSAL AGENT OF A NEW NEMATOSIS OF VARIOUS CROP PLANTS. Jour. Wash. Acad. Sci. 21: 536-538, illus. 1931.

⁸ STEINER, G., and BUHRER, E. M. THE MALE OF THE NEMATODE SPECIES, NEOTYLENCHUS ABULBOSUS STEINER, AND ITS SEXUAL DIMORPHISM. Jour. Wash. Acad. Sci. 22: 482-484, illus. 1932.

SCAB OF GOLDENROD CAUSED BY *ELSINOË*¹

By ANNA E. JENKINS, associate pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture, and H. G. UKKELBERG, in charge of Edison Botanic Garden, Edison Botanic Research Corporation²

INTRODUCTION

The Edison Botanic Garden at Fort Myers, Fla., comprising about 9 acres, was established in 1927 by the late Thomas A. Edison for the purpose of finding a domestic source of rubber. Several thousand plants representing numerous genera and species were gathered from Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Texas, and New Mexico and planted there. Collections also were made in New Jersey, and plants were contributed from many sections of the country by those interested in the project. Contributions of plants indigenous both to this country and to others were made by the United States Department of Agriculture. Goldenrod was found to have a high rubber content and was selected by Edison for further investigations. Most of the other plants were discarded, and by 1934 experimental work was being conducted with goldenrod only. The plants are arranged in beds, 6 by 20 feet, distributed over about 4 acres of the garden, with about 2 feet between the outside rows of any two beds. Each bed contains approximately 168 plants of a single species. The species represented are *Solidago altissima* L., *S. chapmanii* Gray, *S. edisoniana* Small, *S. ellottii* Torrey and Gray, *S. fistulosa* Mill., *S. leavenworthii* Torrey and Gray, *S. mirabilis* Small, *S. nashii* Small, *S. rugosa* Mill., and *S. sempervirens* L.

This paper reports a recent investigation of a hitherto unrecorded disease of goldenrod found in the garden and elsewhere in Florida.

THE DISEASE

NAME

The goldenrod disease, which is caused by an *Elsinoë*, is called "scab" rather than "anthracnose" because of its hyperplastic nature. This is in accordance with a previous suggestion (8)³ that diseases of this character be referred to as "scab."

HISTORY, RANGE, AND SPECIES AFFECTED

Scab of goldenrod was discovered first in the Edison Botanic Garden in June 1933. The disease was noted on young plants and on the young leaves and stems of older plants of *Solidago edisoniana*. Further examination revealed that *S. sempervirens* and *S. fistulosa* also were affected. Lesions on the lower part of the plants indicated that the disease had been present early in the season.

¹ Received for publication July 18, 1935; issued December 1935.

² The writers are indebted to L. G. Polhamus, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Department of Agriculture, for cooperation in various ways, including the examination of goldenrods in certain localities for evidence of the disease. Thanks are also due W. M. Buswell, of the University of Miami, Coral Gables, Fla., for the contribution of specimens of *Solidago* from his private herbarium, on the basis of which certain historical facts relative to the disease were established.

³ Reference is made by number (italic) to Literature Cited, p. 525.

In 1934 lesions were first observed late in April on young plants of *Solidago sempervirens* and somewhat later on *S. fistulosa*. During July the disease appeared in a few beds of *S. edisoniana*, at first affecting only a small number of plants, but gradually spreading throughout these beds and to several others of this species. Young plants transplanted in June and July were severely attacked and some were killed. In one instance, in August, a few lesions were found on plants of *S. leavenworthii* growing in pots in a slat house. These were limited to the leaf bases, regions of the goldenrod plant extremely susceptible to infection. Throughout the season the disease continued to be prevalent and was often severe on *S. edisoniana*, *S. fistulosa*, and *S. sempervirens* (pl. 1; pl. 2, A; pl. 3, A-D). *S. elliotii* and *S. mirabilis* also were moderately attacked.

The distribution of scab on goldenrod growing wild in Florida in Polk, Hardee, De Soto, Charlotte, Lee, and Dade Counties (fig. 1) was established through limited surveys made during the autumn of 1934. At Fort Myers Beach, about 18 miles from Fort Myers, and nearby at Punta Rassa, *Solidago sempervirens* was affected by the disease and in some cases severely so (pl. 4, A and B). Other species, namely, *S. chapmanii*, *S. fistulosa*, *S. stricta* Ait., and *S. tortifolia* Ell., growing in the same locality were not affected.

From Fort Myers northward to Fort Meade, a distance of about 100 miles, *Solidago sempervirens* was found in two different localities near Punta Gorda, and in each case all of the plants were affected. South of Fort Meade was found practically a pure stand of *S. fistulosa* covering approximately 8 acres, and here it was estimated that about 35 percent of the plants were affected. The disease also was present on a few plants of *S. edisoniana* in the vicinity of Wauchula and Fort Meade, the latter being the type locality for the species (14). *S. chapmanii*, *S. minor* (Michx.) Fernald, and *S. tortifolia*, growing in the section surveyed from Fort Myers to Fort Meade, were disease-free.

In Dade County scab was prevalent on *Solidago sempervirens* (pl. 5, I) growing wild in the vicinity of the United States Plant Introduction Garden at Coconut Grove when examined⁴ for the disease in October 1934.

Additional records of scab in Florida, and among them the earliest available, are provided by specimens of *Solidago* from the Buswell collection, which were contributed in order that they might be examined for possible scab lesions. These are present in greater or less abundance on *S. edisoniana* (pl. 2, B), *S. elliotii*, *S. fistulosa*, *S. mirabilis*, and *S. sempervirens* collected in the Edison Botanic Garden on October 31, 1930, December 6, 1933, and intermediate dates, and on wild plants of *S. edisoniana* from Polk County collected on November 9, 10, and 28, and December 3, 1932. A specimen of *S. chapmanii* bearing no date or place of collection in Florida also shows severe infection.

EXPLANATORY LEGEND FOR PLATE 5

A-II, Leaves of *Solidago sempervirens* affected by scab. A, a-c, Upper surface of young leaves; B, a-c, their lower surfaces; C, a and b, upper, and D, a and b, lower surface of two older leaves; C, c, and D, c, lesion penetrating leaf base; E, numerous lesions on upper side of leaf; F, elongate lesion involving midrib; G, leaves dead or dying from scab; H, a, young blighted leaves on an adventitious shoot. (All $\times 1$). *From Edison Botanic Garden, June 21, 1934. I, Scab lesions on wild *Solidago sempervirens* ($\times 1$); J, pycnidium of an unidentified fungus on stem lesions of *S. edisoniana*, Edison Botanic Garden, October 17, 1934 ($\times 475$).

⁴ Examination made by L. G. Polhamus.



Upper part of diseased plants of *Solidago sempervirens* from Edison Botanic Garden, Fort Myers, Fla., June 21, 1934: A, a, and B, a, Regions of extremely severe infection; A, b, adventitious shoots; B, b, leaves dying at the base. \times about $\frac{1}{2}$.



A. Lower part of stems shown in plate 1, or of those from same source; *a*, russeted region. *B.* Scab lesions on young plant of *Solidago radisoniana* representing a phanerogamic specimen from the Edison Botanic Garden collected November 28, 1932. $\times 1$.

The data thus indicate that scab was prevalent on wild goldenrod in Florida for a long period before the Edison Botanic Garden was established. Indirectly the discovery of the disease at the present time may be attributed to the special attention given goldenrod in the garden upon the discovery of its high rubber content, together with the fact that the garden was located within what is now known to be the geographic range of the recently discovered disease. Ecologically this general section is highly favorable to the development of other diseases similar to scab of goldenrod. An example is scab of citrus (*Citrus*), the environmental relations of whose causal fungus (*Sphaceloma fawcettii* Jenkins) have been the subject of considerable investigation (5, 10, 12, 16).

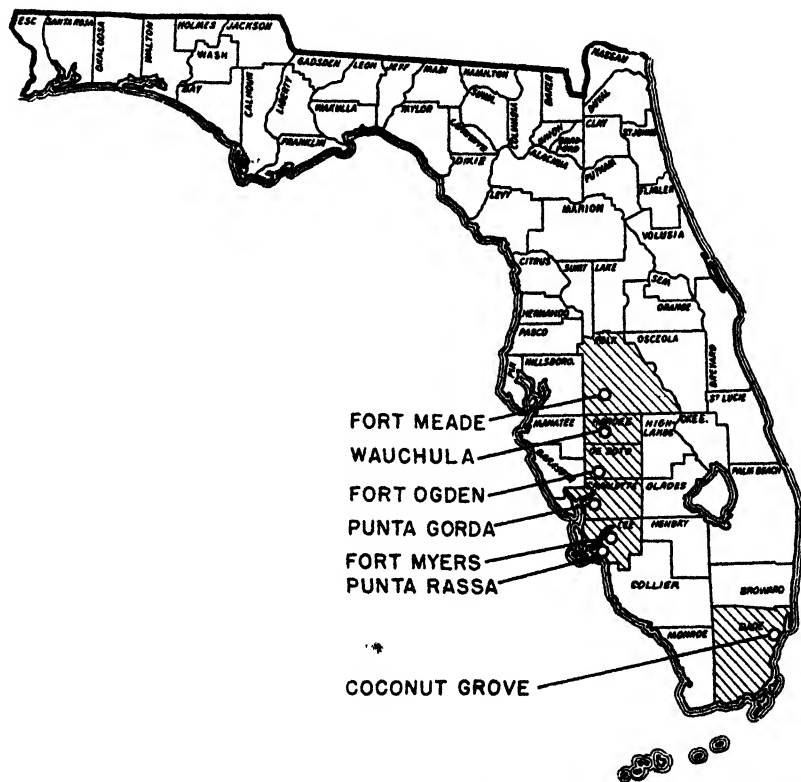


FIGURE 1.—Map of Florida, showing (by shading) the counties in which scab occurs on wild goldenrod, and also the location of towns in or near which the disease was observed.

When examined in July 1934 no scab was found on plantings of goldenrod maintained by the United States Department of Agriculture at Charleston, S. C., and at Savannah, Ga. The plantings at Charleston were again examined for scab early in October,⁵ but no evidence of the disease was found. In the summer of 1935, however, the disease was found on *Solidago sempervirens* at Savannah Beach, Ga., and on this species and *S. fistulosa* growing wild near the Barbour

⁵ Examination made by L. G. Polhamus.

Lathrop Plant Introduction Garden, Savannah, Ga. In the plantings at the garden itself *S. edisoniana*, *S. fistulosa*, *S. leavenworthii*, *S. sempervirens*, and *S. serotina* were all found badly attacked.

IMPORTANCE

Rubber is formed in the leaves of goldenrod (13) and increasingly so as they mature (1). The stunting or killing of young plants by scab results, of course, in a potential loss of leafage, and there is also a direct loss from the killing of the leaves themselves. With respect to *Solidago sempervirens* the loss of leafage from the ravages of the disease is difficult to estimate, because normally some of the leaves die before the plant reaches maturity. Cases have been noted, however, in which most of the leaves were definitely killed by scab. The leafage loss of severely diseased plants of *S. edisoniana* and *S. fistulosa* is estimated to be from 5 to 10 percent. Plants may be severely infected and still remain alive, but whether the rubber content of the diseased leaves is affected has not been determined.

SYMPTOMATOLOGY

MORPHOLOGIC SYMPTOMS

Scab of goldenrod attacks the young growth of the plant above the ground. Young plants may be killed or stunted, as already mentioned, but ordinarily they grow to maturity more or less as usual, the new growth becoming infected as it develops (pl. 1; pl. 2; pl. 3, A-D). Occasionally the terminal bud is killed, preventing further growth of the axis except by the production of an adventitious shoot from below, as sometimes occurs. Some of the leaves wither and die as they unfold, while others that expand later succumb (pl. 1, B, b; pl. 5, G), especially if the highly susceptible basal part (pl. 5, A and B) is affected; in many cases, however, owing to the hyperplastic nature of the disease, the affected leaves are not killed.

Similarly, the stem usually remains alive even though girdled for several centimeters or when as much as four-fifths of its surface is covered with lesions. There may be merely a russetting of the stem (pl. 2, A, a). All or most of the leaves produced during periods extremely favorable for infection may be killed, so that the stem is practically devoid of green leaves for several centimeters (pl. 1, A, a, and B, a). Where the leaves are lost adventitious shoots may be forced into development only to become infected in turn (pl. 1, A, b; pl. 5, H).

ON *SOLIDAGO SEMPERVIRENS*

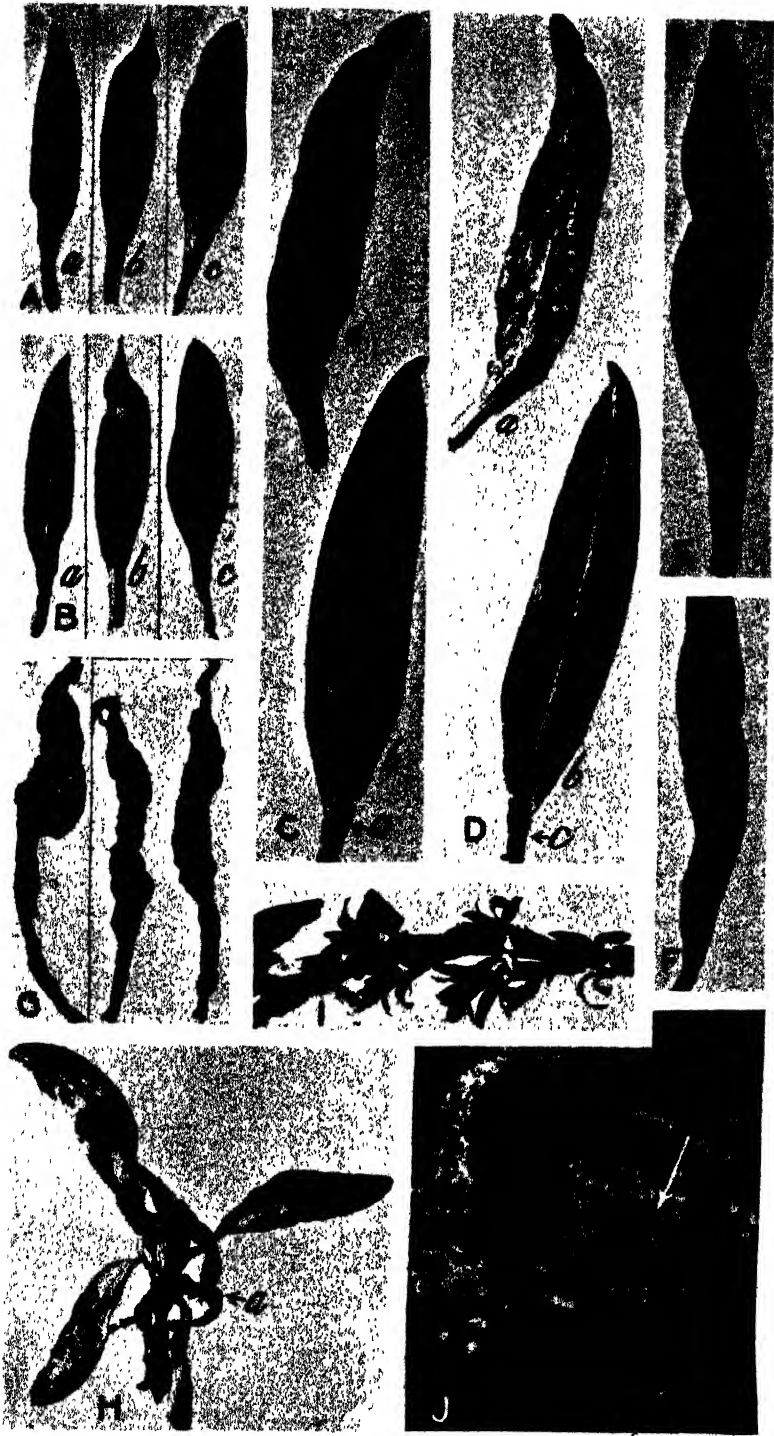
The leaf spots on the highly susceptible species of *Solidago sempervirens* are larger and more striking, as well as often more numerous, than on the other species. They occur anywhere on either side of the leaf (pl. 5, A-F), although somewhat more frequently on the lower surface. Midribs, veins, and petioles as well as the basal part of apetiolate leaves are often affected (pl. 4; pl. 5, A-D; pl. 6, B, a). Lesions on the bases of apetiolate leaves (pl. 5, C, c, and D, c) and those occurring elsewhere on large succulent leaves (pl. 6, B) may discolor both leaf surfaces, but generally they are visible on one leaf surface only (pl. 5, A, a, b, c, and B, a, b, c; C, a, and D, a; C, b, and D, b). Lesions may extend for a greater or less distance from the base

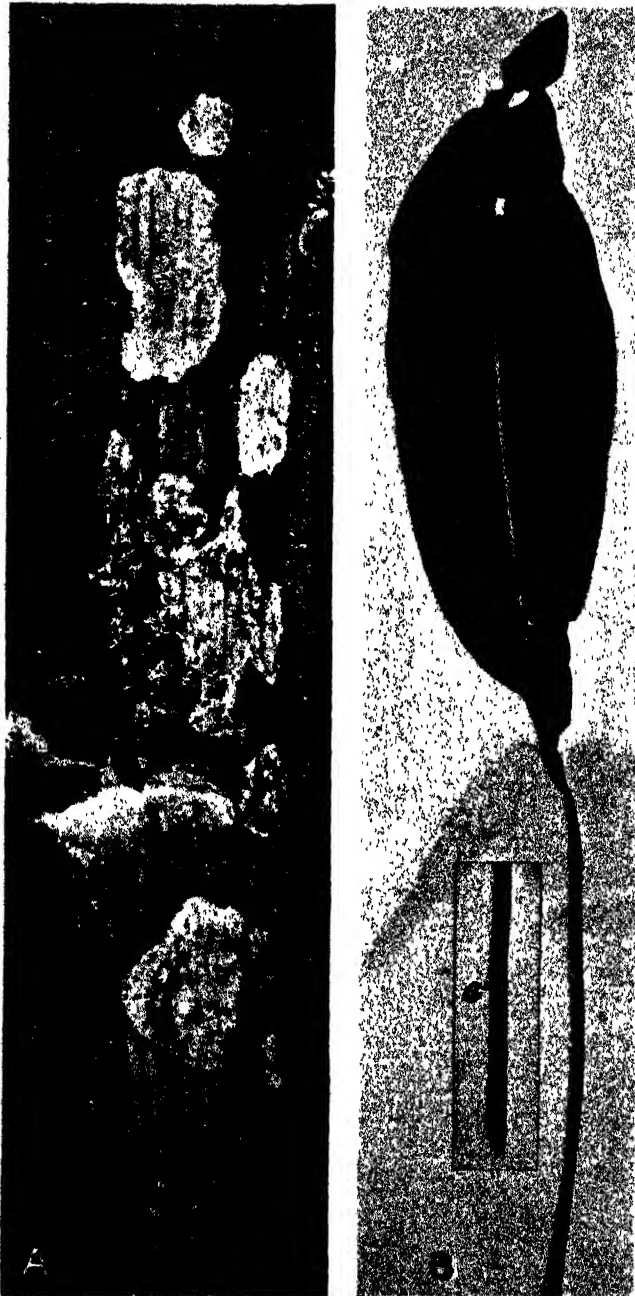


Scab lesions on (A) leaves of *Solidago fistulosa*, (B) on stem of *S. edisoniana*, and on (C) stem and (D) inflorescence leaves of *S. sempervirens*. Material from Edison Botanic Garden, A-C collected August 4 and D October 17, 1934. E-G, Agar slant cultures of the *Elsinoë* causing the disease, approximately a month old, on (E) potato dextrose, (F) corn meal, and (G) beef. All $\times 94\times$. Drawings by J. Marion Shull.



Wild *Solidago sempervirens* affected by scab: A From Punta Rasa, August 17, 1934; B from Fort Myers Beach, August 14, 1934. A, a, Base of stem; B, a, upper part of same plant. Photographs from dry specimens. $\times 1$.





, a, Ascomata on stem lesions of *Solidago sempervirens* ($\times 7$); B, blade and (a) petiole lesions on the same species resulting from artificial inoculations with the *Elsinoë* ($\times 1$).

toward the apex of the leaf (pl. 4); those on the lower or dorsal leaf surface may be continuous with stem lesions. The more restricted leaf lesions range from punctiform areas to circular, subcircular, or irregular spots reaching 5 mm in diameter (pl. 4; pl. 5, *A-F*). Lesions may be slightly raised at first, and later depressed on one leaf surface and bulged on the other (pl. 5, *A, b*, and *B, b*). The entire leaf may be bent away from the point of infection. In the beginning the lesions appear as brown, often "madder brown",⁶ water-soaked spots. The center and soon the entire lesion, or all except a narrow bordering line or edge, becomes "vinaceous buff." This coloration finally becomes white or gray.

On stems of *Solidago sempervirens* the lesions are of essentially the same coloration as on the leaves. Sometimes they are surrounded by a narrow dark line, and they may be slightly raised, especially when old (pl. 3, *C*). The smaller spots are often circular, subcircular, or irregular, and $8 \pm$ mm in diameter; occasionally they are more or less linear. Larger lesions, sometimes formed by the fusion of smaller spots, are often extremely irregular in shape. They may be unevenly scalloped or variously lobed, and they sometimes enclose green patches of the stem. Transverse cracks may appear on the surface of fairly young lesions still "vinaceous buff" in color (pl. 2, *A*).

ON *SOLIDAGO EDISONIANA* AND OTHER SPECIES

Although similar to those of *Solidago sempervirens*, the scab lesions on *S. edisoniana* differ in certain respects. The leaf spots are often circular to subcircular, 2 to 3 mm in diameter (pl. 2, *B*). They are generally reddish at first, then more or less permanently "hazel" or "brick red", although in some cases they are paler or even white. Sometimes they discolor both leaf surfaces. The leaf spot may become perforated or fall entirely away, or only the cuticle on one leaf surface may remain. The stem lesions are similar to the leaf lesions (pl. 3, *B*), but when old they may be somewhat paler and bordered by purple.

The lesions of *Solidago elliotii* and *S. mirabilis* are similar to those of the closely related species *S. edisoniana*, while those of *S. fistulosa* (pl. 3, *A*) are somewhat intermediate between those of *S. edisoniana* and *S. sempervirens*.

SIGNS

The signs of goldenrod scab are primarily the fruit bodies of the perfect stage of the pathogen, which may be relatively inconspicuous. These ascomata are present from as early as June until the end of the season, and they appear as small dark more or less convex areas (pl. 6, *A, a*) representing the epithelial covering (pl. 7, *B, a*, and *F, b*). For the most part they are confined to mature or old stem and leaf-base lesions. Small black specks occasionally present on old lesions (pl. 5, *J*) are pycnidia or pycnidialike structures, but it has not yet been possible to determine definitely whether they represent a conidial stage of the causal fungus. In some cases mature lesions are darkened by a thin layer of superficial dark hyphae or other vegetative development of the fungus (pl. 7, *A*).

⁶ The color readings given in quotations are by J. Marion Shull and are based on the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

HISTOLOGIC SYMPTOMS

As in related diseases, the lesions are characterized by the formation of what has been referred to by Butler (3, pp. 180-181) as "wound cork." This development in leaf lesions of citrus scab has been described in detail by Cunningham (4). Based on Cunningham's account and on prepared mounts which he gave Butler, the latter (3, p. 181, and pl. 8, figs. 6 and 7) has summed up the situation as follows:

The pathogen—

causes a necrosis of a few of the superficial cells of the leaf and provokes the active division of the spongy parenchyma. The new walls are roughly parallel, in the early stages, to the necrosed surface and the process extends to a considerable depth, sometimes leading, in attack on the under surface, to divisions in the palisade cells on the opposite side. Near the lesion the cells elongate more towards the injured part and become more divided than those farther away, and in the layer of long cells with several cross walls a cork cambium eventually develops * * *. This extends up to the epidermis all around the lesions and cuts off cork on its outer side and a little phelloderm on the side toward the sound tissues. All the primary cell walls of the hyperplastic area are thickened and intercellular spaces are much reduced.

THE CAUSAL FUNGUS

CLASSIFICATION

The ascomycete causing scab of goldenrod is a previously unreported member of the genus *Elsinoë* Rac., and its imperfect stage is typical of the form genus *Sphaceloma* De Bary. As previously shown (6), this genus belongs to the Myriangiales, which are typified by *Myriangium duriaei* Mont. and Berk. *Elsinoë* is typified by *E. canavaliae*, which causes scab of swordbean (*Canavalia gladiata* (Jacq.) DC.). A closely related and actually better known species, however, is *E. phaseoli* Jenkins (2), in connection with the study of which certain new features pertaining to the morphology of the genus (7, 9) were demonstrated, such as the double-walled character of the ascus (7). Available literature relative to the history and relationships of the order were reviewed by the writer in connection with the discussion of *E. canavaliae* (6). Tai (15) has since discussed the relationships of the order on the basis of his study of the development of *Myriangium bambusae* Rick. He interprets the outer sheath or primary wall of the ascus as homologous with the perithecial wall in the Sphaeriales and also with the rudimentary wall (11) of the ascocarp in *Catacauma flabellum* (Schw.) Theiss. and Syd. of the Dothideales.

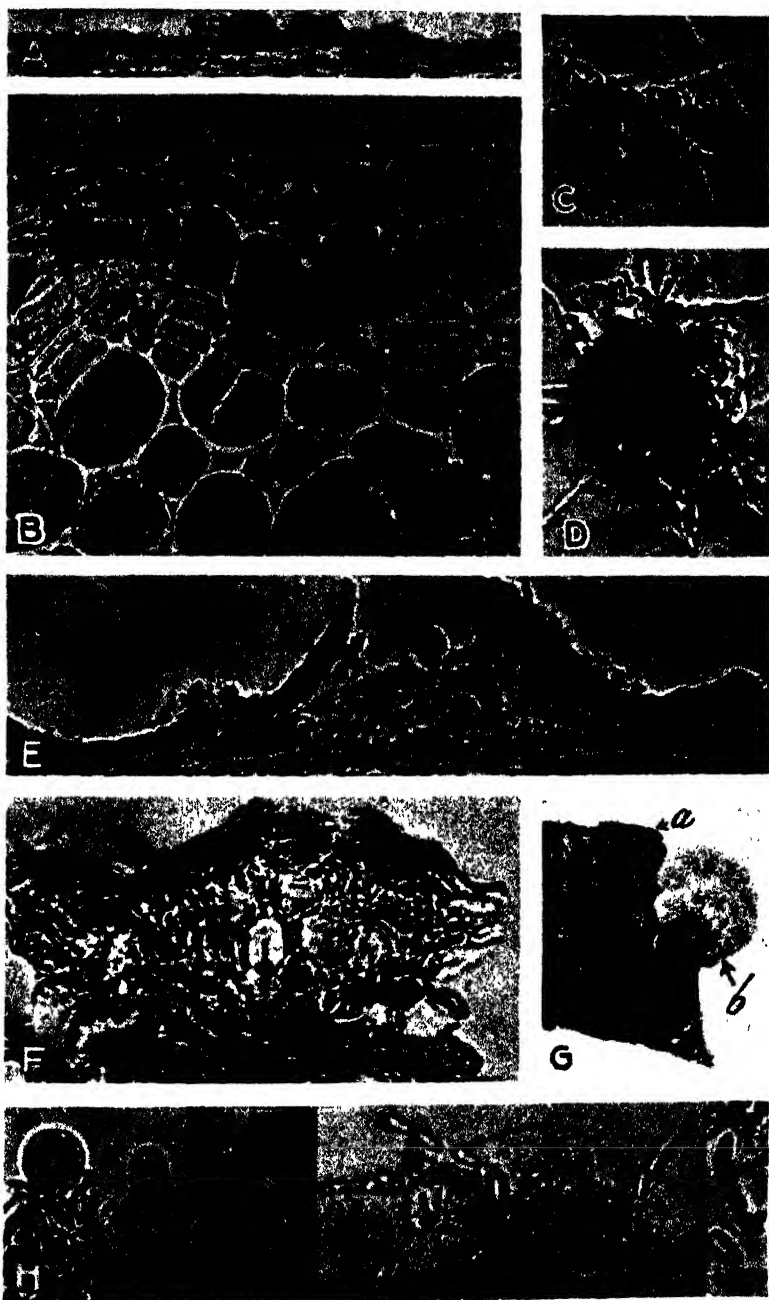
MORPHOLOGY

PERFECT STAGE

Rather recently formed ascomata (pl. 7, B) of the goldenrod *Elsinoë*, some containing as many as 15 asci, were present on a few of the oldest lesions of the upper 20 to 25 cm of stems collected in the

EXPLANATORY LEGEND FOR PLATE 7

Elsinoë solidaginis: A, Surface of old stem lesions darkened by superficial growth of the fungus; a, possibly an endospore. B, Section of a lesion shown in plate 6, A, showing a young ascoma; a, epithecium; b and c, asci; d, hyperplastic area of lesion ($\times 375$). C and D, Production of conidia in young culture from an old hyphal fragment and from a small mycelial mass; C, a, conidiphore; C, b, and D, a, conidia; D, b, young hyphae ($\times 400$). E-F, Sections of outer part of stem lesions showing more mature ascomata than the ascoma represented in B; E, a, cavity or pocket containing hyaline spherical conidia; E, b, epithecium; E, c, asci. (E $\times 350$; F $\times 475$). G, Tissue planting (a) and culture growing from it ($\times 10$). H, maceration of ascomata from stem lesion showing; a, ascus, with both walls intact, although line of outer wall is not visible in the photograph; asci whose inner elastic walls (b) have expanded following rupture of the (c) outer inelastic walls; d, ascospores within ascus; e, still greater expansion of inner part of ascus, from whose apices several spores have been discharged; (f), 1- and 2-septate ascospores (a $\times 440$; c $\times 450$; e $\times 330$; f $\times 440$.)



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

Edison Botanic Garden on June 1, 1934 (pl. 6, *A, a*). Older ascomata from specimens collected in August are shown in plate 7, *E* and *F*, and asci and ascospores in plate 7, *B, b* and *c*; *E, c*; and *H*. *H, a*, represents an ascus of which the outer sheath is probably about to rupture; *H, b*, partial expansion of the ascus after the rupture of this sheath; and *H, d-f*, ascospores still within the expanded asci or discharged from them.

A feature of the ascospore of *Elsinoë*, first observed in the case of *E. phaseoli*⁷ and also noted on the goldenrod *Elsinoë*, is the rupture of the wall of the ascospore allowing the protoplasmic contents of one or more cells to escape in sporelike masses.

CONIDIAL STAGE

Tufts of hyaline conidiophores representing the *Sphaceloma* stage of the goldenrod fungus were found in certain sections of young "madder brown" lesions on *Solidago sempervirens*. These evidently were produced from more or less horizontal subcuticular or intraepidermal hyphae after the manner illustrated in plate 7, *C*, showing conidial formation within a 24-hour period, from a fragment of an old hypha. The culture was made by smearing small masses of an old culture on the surface of a thin film of corn-meal agar on a glass slide. To provide a moist atmosphere, the new culture was placed in a Petri dish lined with filter paper. Plate 7, *D*, shows additional new conidia (*a*) and also a young hypha (*b*) produced from a small mycelial mass transferred to the fresh agar substrate. There was soon conidial formation from the new hyphae, the conidia in some instances being sessile on the hyphae. The conidia just described were generally ovoid or elongate-elliptical, and often biguttulate, measuring 6.5 to 8.6 μ by 2.6 to 4 μ . Some of the conidia germinated practically as soon as formed, either by the production of germ tubes or sprout conidia. Spherical conidia at first minute but enlarging to at least 3 μ in diameter occur singly or in agglutinated masses. Apparently they are produced from almost any structure of the fungus, and as here illustrated (pl. 7, *E, a*) they appear to have formed within the epithecium. The spherical body shown in plate 7, *A, a*, is possibly an endospore.

The dark, membranous pycnidium apparently of the *Elsinoë* shown in plate 5, *J*, was produced subepidermally; the conidia (*a*) and conidiophores (*b*) within it resemble those shown in plate 7, *C* and *D*. Other more or less similar membranous structures, often lenticular in shape, were seen in or on the lesions.

IDENTITY

It appears that an *Elsinoë* has not been reported on goldenrod or other Compositae heretofore. The asci and ascospores are somewhat smaller than those of other species that have been described, and the organism is culturally distinct from the members of this genus and of *Sphaceloma* so far as isolated. The name *Elsinoë solidaginis* is therefore proposed for the fungus, and the following technical description is given.

⁷ Unpublished data.

TECHNICAL DESCRIPTION

Elsinoë solidaginis, n. sp.

Hypae subcuticular, intraepidermal, or subepidermal, i. e., in the tissue outside of the wound cork, hyaline, or dark, sometimes chainlike, up to at least 6μ in diameter, sometimes forming more or less effuse stromata reaching 15μ in thickness; ascomata apparently intraepidermal for the most part, punctiform, reaching 150μ in surface diameter and 50μ in thickness; dark-colored epithecium up to 20μ thick, becoming ruptured and exposing underlying ascogenous layer; unruptured asci mostly spherical to obpyriform, 15 to $17\mu \times 15$ to 18μ , occasionally with a slight stipe; ascospores colorless, 2- to 3-celled, 8 to $13\mu \times 4$ to 5μ , protoplasmic cellular contents sometimes escaping as sporelike granular masses; conidial stage of the form genus *Sphaceloma*, conidiophores as observed in culture often more or less conical, 1- to 2-septate, bearing conidia acrogenously, sometimes several in succession; conidia ovoid to oblong-elliptical, hyaline, often biguttulate, 1-celled, 6.5 to $8.6\mu \times 2.5$ to 4μ , germinating by means of a germ tube or by the production of sprout conidia; spherical conidia produced from various structures of the fungus, of various sizes and with thick, viscid covering, often forming agglutinated masses.

Ascomata punctiformia, usque 150μ diam. ad superficiem et 50μ in crassitudine; asci infracti sphaerici vel obpyriformes, 15 – $17\mu \times 15$ – 18μ ; ascosporae 2–3 cellulares, 8 – $13\mu \times 4$ – 5μ ; status conidiophorus ad formam generem *Sphacelomatem* pertinens; conidiophora (in culturis) saepe plus minusve conica 1–2-septata; conidia acrogena, ovoidea vel oblongo-elliptica, unicellularia, 6.5 – $8.6\mu \times 2.5$ – 4μ ; conidia sphaerica usque 3μ diam., e structuris variis fungi nata, massas agglutinatas formantia.

Hosts.—On wild and domesticated species of *Solidago chapmanii*, *S. edisoniana*, *S. elliotii*, *S. fistulosa*, *S. leavenworthii* (rarely), *S. mirabilis*, and *S. sempervirens*, producing the disease known as scab, hyperplastic in nature, infecting tender young growth, sometimes killing it, but often producing more or less extensive diseased areas on the surface of stems and leaves; lesions of various sizes and shapes, on *S. sempervirens* generally "vinaceous buff", becoming white or nearly so; on *S. edisoniana*, *S. elliotii*, and *S. mirabilis*, often "hazel" to "brick red", occasionally white or whitish; on *S. fistulosa* of intermediate coloration.

Distribution.—Florida: Polk, Hardee, De Soto, Charlotte, Lee, and Dade Counties.

Specimens examined.^a—Florida: On *Solidago chapmanii*, W. M. Buswell (date not given).^b

On *S. edisoniana*, Hardee Co., Wauchula, H. G. Ukkelberg, Sept. 30, 1934; Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Nov. 28, 1932*, Aug. 17 and Nov. 10*, 1933; H. G. Ukkelberg, July 9, Aug. 4 (type), and Oct. 17, 1934; Feb. 6, 1935; Polk Co., W. M. Buswell, Nov. 9*, 10*, and 28*, and Dec. 3*, 1932; Fort Meade, H. G. Ukkelberg, Sept. 30, 1934.

On *S. elliotii*, Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Dec. 4* and 6*, 1932, and Dec. 6, 1933*.

On *S. fistulosa*, De Soto Co., Fort Ogden, H. G. Ukkelberg, Sept. 20, 1934; Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Nov. 10, 1930*, and May 10, 1933*; H. G. Ukkelberg, Aug. 4 and Oct. 17, 1934; Feb. 6, 1935.

On *S. mirabilis*, Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Dec. 4, 1933*.

On *S. sempervirens*, Charlotte Co., Punta Gorda, H. G. Ukkelberg, Sept. 30, 1934; Dade Co., Coconut Grove, L. G. Polhamus, Oct. 1934 (specimen represented by photographs); Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Oct. 31, 1930*; H. G. Ukkelberg, June 1, 21, July 9, and 28, Aug. 4, Oct. 17, and Oct. 26 (infection from artificial inoculation in greenhouse), 1934; Feb. 6, 1935; vicinity of Fort Myers, W. M. Buswell, Oct. 24, 1931*; H. G. Ukkelberg, Aug. 14, 1934 (beach); Punta Rassa, H. G. Ukkelberg, Aug. 17, 1934.

District of Columbia: On *S. sempervirens*, A. E. Jenkins, Oct. 12, 1934 (infection from artificial inoculation) in laboratory.

^a The specimens examined are deposited in the mycological collections of the Bureau of Plant Industry, U. S. Department of Agriculture, and a living culture of the fungus (culture no. 430 A. E. Jenkins) has been sent to the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

^b The specimens marked by an asterisk represent specimens from the Buswell collection on which lesions of scab were found during the course of the present study.

CULTURAL CHARACTERISTICS

Pure cultures of the fungus were obtained from isolations made from brown lesions on young leaves of *Solidago sempervirens* collected in the Edison Botanic Garden on July 6. For the first isolations, made on July 11, scrapings from the surface of the lesions were plated on corn-meal agar. By July 25 several thalli characteristic of the genus *Elsinoë* were visible in the six plates that were made. The circular, glistening growths were just becoming colored. The largest thallus, measuring about 2 mm in diameter, was transferred to a potato-dextrose agar slant. When observed about a week later it had increased in size to about 3 mm. The margin was then raised and was "pinkish cinnamon" in color; the central region was depressed and was "cinnamon" in color.

On July 12 additional isolations were made from the specimens collected on July 6 by dipping pieces of young leaves and stems in mercuric chloride (1:1000), rinsing them in sterile water, and then planting them on the surface of corn-meal agar plates. Plate 7, G, shows one of the thalli obtained from these plantings.

More abundant cultural growth was obtained by cutting goldenrod stems into short lengths, treating them as for the leaf plantings, and then transferring them to test tubes containing a small amount of sterile water. When examined after 3 weeks some of the lesions were covered with a mat of fungus, consisting chiefly of conidia, including swollen and germinated conidia of various sizes. Transfers of this growth failed to yield pure cultures, but platings would doubtless have given many pure thalli of the fungus.

In a set of parallel cultures of the *Elsinoë* grown for approximately a month on agar slants of potato dextrose, corn meal, and beef, the coloration of the fungus was generally "maroon" to nearly black, "bay", and "clay color", respectively. Similar cultures on glycerin agar slants were "pale drab-gray" at the center and "maroon" to black in the peripheral region.

INOCULATION EXPERIMENTS

At the Edison Botanic Garden artificial inoculations were made on September 20 on young potted plants in a greenhouse in which the temperature was not controlled. The species inoculated were *Solidago altissima* and *S. leavenworthii*, which are highly resistant or immune to scab, and the two highly susceptible strains *S. fistulosa* and *S. sempervirens*. *S. sempervirens* is more susceptible than *S. fistulosa*, as already explained, and the two strains of these species employed were selected for their extreme susceptibility. The inoculum consisted of the culture isolated from *S. sempervirens* on July 11, grown for several weeks on potato-dextrose agar.

Several inoculations were made on each species or strain by the cotton-plaster method employed by Winston (16, p. 23) in similar inoculations, i. e., the cultural growth was macerated and placed on young leaves with the fungus in contact with the leaf. The whole was then covered with paraffin-paper hoods gathered and tied in such a way as to prevent the drying out of the cotton pads. The inoculated growth was then tied to a stake to hold it in position and to prevent the weight of the wet cotton from breaking the plant. The

paraffin paper and the cotton bearing the inoculum were removed after 48 hours.

In another experiment a few plants of each of the species and strains mentioned were atomized with a water suspension of a culture and held for 48 hours in a moist chamber before being removed to the greenhouse bench. In this experiment, as in the other, the check plants received the same treatment as those sprayed except that no inoculum was used.

The conditions of the experiments were apparently rather unfavorable for infection for, so far as could be detected, scab lesions were produced only on *Solidago sempervirens* inoculated by the cotton-plaster method (pl. 6, B). The lesions were small and "madder brown" when first observed on October 9. By October 20 they were "vinaceous buff" and the largest were 5 mm in diameter.

At Washington, D. C., under weather conditions believed to be favorable for infection, young inflorescence leaves inoculated by the cotton-plaster method became infected chiefly on the leaf bases. The checks remained healthy.

CONTROL

In the garden one bed of *Solidago sempervirens* was sprayed with bordeaux mixture (4-4-50) during May and June. Three applications were made at 2-week intervals, with no apparent checking of the disease. During July, August, and September, lime-sulphur was applied weekly on a series of six beds of *S. edisoniana*. In this case the amount of the disease was not reduced at first, but in the latter part of the experiment partial control was obtained. In order to obtain control by spraying, frequent applications of a protectant from practically the beginning of the growing season probably would be necessary.

It appears that selection for resistance may prove an effective means of control. Variation in the degree of susceptibility of different clonal lines obtained from rootstock cuttings was observed in 1934, particularly in the case of *Solidago edisoniana*. For example, plants of the same clones were heavily infected while other clones in nearby rows were free from lesions, or nearly so, although they were in contact with heavily infected growth. In some instances strains of *S. fistulosa* and *S. sempervirens* introduced into the garden from different localities and differing somewhat in vegetative characters have exhibited different degrees of susceptibility. The Edison selection *S. leavenworthii* is highly resistant, as previously indicated.

SUMMARY

Scab of goldenrod, hitherto unreported, was discovered in the Edison Botanic Garden, Fort Myers, Fla., in June 1933. Limited surveys made in Florida during the autumn of 1934, together with an examination of herbarium specimens, revealed the fact that the disease was present in the garden as early as 1930, and that it occurs on wild goldenrod in Polk, Hardee, DeSoto, Charlotte, Lee, and Dade Counties. The species known to be susceptible are *Solidago chapmanii*, *S. edisoniana*, *S. elliotii*, *S. fistulosa*, *S. leavenworthii*, *S. mirabilis*, and *S. sempervirens*. Of these, *S. edisoniana*, *S. fistulosa*, and *S. sempervirens* are highly susceptible and *S. leavenworthii* slightly so.

The disease results, directly or indirectly, in the loss of the leafage in which rubber is formed.

The symptoms of the disease are described.

The causal fungus belongs to the genus *Elsinoë*. The morphology and cultural characteristics of the organism are given, and it is described as a new species, *E. solidaginis*. Artificial inoculations on *Solidago sempervirens* gave positive results.

LITERATURE CITED

- (1) ANONYMOUS.
1934. RUBBER PLANT EXPERIMENTS. Science (n. s.) 80: 261-262.
- (2) BRUNER, S. C., and JENKINS, A. E.
1933. IDENTITY AND HOST RELATIONS OF THE ELSINOË OF LIMA BEAN. Jour. Agr. Research 47: 783-789, illus.
- (3) BUTLER, E. J.
1930. SOME ASPECTS OF THE MORBID ANATOMY OF PLANTS. Ann. Appl. Biol. 17: [175]-212, illus.
- (4) CUNNINGHAM, C. H.
1928. HISTOLOGY OF THE LESIONS PRODUCED BY SPHACELOMA FAWCETTII JENKINS ON LEAVES OF CITRUS. Phytopathology 18: 539-545, illus.
- (5) FAWCETT, H. S.
1921. SOME RELATIONS OF TEMPERATURE TO GROWTH AND INFECTION IN THE CITRUS SCAB FUNGUS CLADOSPORIUM CITRI. Jour. Agr. Research 21: 243-253.
- (6) JENKINS, A. E.
1931. SCAB OF CANAVALLIA CAUSED BY ELSINOË CANAVALLIAE. Jour. Agr. Research 42: 1-12, illus.
- (7) ———
1931. LIMA-BEAN SCAB CAUSED BY ELSINOË. Jour. Agr. Research 42: 13-23, illus.
- (8) ———
1933. APPLICATION OF THE TERMS "ANTHRACNOSE" AND "SCAB" TO DISEASES CAUSED BY SPHACELOMA AND GLOEOSPORIUM. Phytopathology 23: 389-395, illus.
- (9) ———
1933. FURTHER STUDIES OF LIMA-BEAN SCAB. Phytopathology 23: 662-666, illus.
- (10) ——— and FAWCETT, H. S.
1933. RECORDS OF CITRUS SCAB MAINLY FROM HERBARIUM SPECIMENS OF THE GENUS CITRUS IN ENGLAND AND THE UNITED STATES. Phytopathology 23: 475-482, illus.
- (11) ORTON, C. R.
1924. STUDIES IN THE MORPHOLOGY OF THE ASCOMYCETES. I. THE STROMA AND COMPOUND FRUCTIFICATION OF THE DOTHIIDEACEAE AND OTHER GROUPS. Mycologia 16: 49-95, illus.
- (12) PELTIER, G. L., and FREDERICH, W. J.
1924. RELATION OF ENVIRONMENTAL FACTORS TO CITRUS SCAB CAUSED BY CLADOSPORIUM CITRI MASSEE. Jour. Agr. Research 28: 241-254, illus.
- (13) POLHAMUS, L. G.
1933. RUBBER CONTENT OF VARIOUS SPECIES OF GOLDENROD. Jour. Agr. Research 47: 149-152.
- (14) SMALL, J. K.
1933. MANUAL OF THE SOUTHEASTERN FLORA; BEING DESCRIPTIONS OF THE SEED PLANTS GROWING NATURALLY IN FLORIDA, ALABAMA, MISSISSIPPI, EASTERN LOUISIANA, TENNESSEE, NORTH CAROLINA, SOUTH CAROLINA, AND GEORGIA. 1554 pp., illus. New York.
- (15) TAI, F. L.
1931. OBSERVATIONS ON THE DEVELOPMENT OF MYRIANGIUM BAMBUSAE RICK. Sinensia Contrib., Metropolitan Mus. Nat. Hist. Acad. Sinica. Nanking 1: [147]-164, illus.
- (16) WINSTON, J. R.
1923. CITRUS SCAB; ITS CAUSE AND CONTROL. U. S. Dept. Agr. Bull. 1118, 39 pp., illus.

OXIDATION AND GAS FORMATION IN THE SPONTANEOUS HEATING OF HAY¹

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INTRODUCTION

In a series of large-scale experiments on the spontaneous heating of hay, conducted by the Bureau of Chemistry and Soils, hay was stored under varying conditions in order to determine those conducive to excessive heating and ultimately to spontaneous ignition, as well as to investigate the causes and effects of spontaneous heating under these conditions.

In these experiments the progress of heating was followed carefully, observations of various factors that could influence spontaneous heating were made, and the products of such heating were investigated.

The results of analyses of the gases formed during the heating of hay in these experiments showed a striking similarity to the results of a laboratory study designed to determine the relative tendency of undecomposed and decomposed hay to absorb oxygen. For this reason both investigations are presented in this paper.

Conflicting hypotheses have been proposed by investigators to account for the spontaneous heating and ignition of hay. However, it is commonly accepted that the production of heat in a mass of undercured or wet hay at temperatures up to 70° C., or slightly higher, is due mainly to the respiration process of the living plant cell and the activity of micro-organisms. But to account for temperatures above this, at which micro-organisms are no longer active, causes other than biological are ascribed. These are generally referred to as chemical.

The extent to which heating during the period of biological activity may be ascribed to purely chemical action has been the subject of considerable investigation and debate. It has been held by proponents of chemical-activity theory that if the higher temperatures are to be ascribed to chemical or physical agencies, it is quite reasonable to suppose that these agencies already have begun to operate at the lower temperatures. Since the results presented in this paper are concerned primarily with temperatures lying within or slightly above the range ascribed to biological agencies, a brief review of investigations supporting the theory of chemical action at these temperatures is given as a basis for the discussion of the results.

¹ Received for publication June 5, 1935, issued December 1935.

² This paper includes the results of 1 phase of the general investigation of the spontaneous heating and ignition of agricultural products conducted by the Chemical Engineering and Food Research Divisions, Chemical and Technological Research. The author acknowledges with thanks his indebtedness to C. A. Browne, principal chemist in charge of research; to D. J. Price, principal engineer in charge, Chemical Engineering Division; and to his associates in the conduct of the large-scale spontaneous-heating experiments, for the use of data pertaining to these experiments. To H. E. Roethe, engineer; M. A. Bradshaw, assistant engineer; and E. D. Gordon, formerly assistant engineer, Chemical Engineering Division, the author is indebted for assistance in the collection of the gases investigated.

REVIEW OF PREVIOUS INVESTIGATIONS

Miehe (6, 7)³ held that beyond the limited effect of plant respiration the rise of temperature is due mainly to the action of micro-organisms. This action could carry the temperature up to 70° C., or slightly higher, but he found that at 68.5° all micro-organisms had been destroyed. He opposed the view that ordinary heating in haymows is due to simple chemical activity, but suggested that when the temperature rose above approximately 70° some physical or chemical change had been produced by the long-continued effect of heat or bacterial action at 70°, which rendered the hay susceptible to purely chemical oxidation. He suggested further that hay carbonization might occur by a process of dry distillation even at a temperature as low as 70° to 80°, if this temperature were maintained for a considerable time.

According to Burri (3), in the processes of spontaneous heating the temperature rarely exceeds 70° C., beyond which the activity of enzymes and micro-organisms is absolutely excluded. If the temperature can be raised beyond 70° by purely chemical processes it would appear plausible that the same agencies are already active at lower temperatures, even between 50° and 70°.

He held that there were good reasons for believing that, in the case of hay which is sufficiently wet, and in which the living plant has died and the respiration process has ceased with the production of a temperature of 45° to 50° C., a further increase in temperature can be produced by a chemical oxidation process, but that this chemical process alone would probably not be of sufficient intensity and duration to raise the temperature to 70°. However, on the assumption that in haymaking the respiration enzymes have been rendered more resistant to heat than is commonly supposed, the heat formation resulting from the respiration process probably would cease, not at 45° to 50°, but perhaps at 60° or higher. On this assumption a temperature level might be reached without the cooperation of micro-organisms beyond which purely chemical and physical processes would account for the heat production.

Boekhout and DeVries (1) concluded from their investigations of the heating of hay and tobacco that micro-organisms have no part in the phenomenon, but that spontaneous heating of hay is only a chemical oxidation process in which iron occurring naturally in the plant acts as a catalyzer.

Haldane and Makgill (4) determined the relations of oxygen absorption and carbon dioxide liberation in samples of hay through which a constant flow of pure air was passed. They confirmed the biological conclusions of Miehe and concluded further that even at temperatures as low as 38° to 41° C. a simple chemical oxidation takes place side by side with the biological oxidation. The chemical oxidation could be distinguished easily from the biological by the fact that the former diminished with lapse of time, and by the ratio of carbon dioxide liberated to the oxygen consumed. This ratio was always considerably below unity in the chemical oxidation and about equal to or greater than unity in the biological oxidation.

At 71° C. there was an enormous fall in the oxidation rate, since all microbial activity was suspended and only the chemical oxidation

³ Reference is made by number (Italo) to Literature Cited, p. 546.

continued. A further rise of temperature to 81° caused a great increase in the chemical oxidation rate, and a similar result occurred at 90°. According to Haldane and Makgill, the rapid development of heat in a large mass of damp hay is due almost entirely to the growth and activity of micro-organisms, which can raise the temperature to about 70°, but oxidation occurs in the damp hay even when microbial activity is absent.

Truninger (8), after discussing oxygen respiration and intramolecular respiration of plants and the role of bacteriological activity in heat production, suggested the desirability of investigating whether the activity of certain bacteria and molds in the fermenting haystack may produce easily oxidizable substances which might serve as the source of subsequent purely chemical processes.

The theory of the spontaneous heating and ignition of large masses of hay proposed by Browne (2) is based upon the preliminary production by micro-organisms under more or less perfect anaerobic conditions of unsaturated, highly unstable, intermediate-fermentation products upon the surfaces of the porous, cellular materials. The duration of existence of these readily oxidized products depends upon the quantity of air that gains access to the fermenting mass of hay and also upon the quantity of moisture present as a reacting medium. If the air has free access these compounds are destroyed almost as soon as formed, with the result that when the vegetative micro-organic life is all destroyed, at 70° to 80° C., there is not a sufficient residue of such easily oxidizable substances to carry the heat production to higher limits. The heat of the microbial life period is probably due in large measure to the oxidation of the same unstable products that participate in raising the temperature above 80°.

LARGE-SCALE EXPERIMENTS

PROCEDURE

The experiments reported here comprise 5 of the 10 spontaneous heating experiments completed in the experimental hay barn on the United States Animal Husbandry Farm at Beltsville, Md. A brief statement regarding each experiment follows. In all the experiments alfalfa hay containing less than 30-percent moisture is referred to as cured alfalfa.

EXPERIMENT 1, JUNE 1929

The hay stored in experiment 1 was 14 tons of undercured alfalfa, containing a minimum of 30.69 and a maximum of 43.45 percent moisture, an average of 36.35 percent for seven levels of mow, 1, 3, 6, 7, 8, 11, and 12 feet from the floor of the barn. The hay was stored on June 7 to a height of 12 feet in a corner section of the barn, 16 by 16 feet, leaving the east and south sides of the mow exposed. A maximum temperature of 72° C. was reached on June 15, in the center of the mow. After June 16 the temperature throughout the mow continued to drop until, on August 27, the average of the five highest temperatures was 41°. The final height of the mow was 8 feet at the center.

EXPERIMENT 3, JULY 1930

In experiment 3, as in all others following this, the entire floor space of the barn (26 by 24 feet) was used for storage, except for a narrow alleyway (3 by 24 feet) on the entrance, or east side of the barn, thus

exposing the mow on only one side. The hay used was 55,987 pounds of baled alfalfa, having an average moisture content of 15.23 percent. As the hay was put into the mow, it was torn apart and sprinkled with 40,000 pounds of water, bringing its moisture content up to an average of 50.55 percent. The barn was filled to a height of 15 feet on July 22 to 25. The final settled height of the mow was 13 feet. A temperature of 87.5° C. was reached on July 30 at thermocouple 20, located 6½ feet from the floor, 8 feet directly east of the center of the mow, and 4 feet from the alleyway on the east. On August 20 the maximum temperature, 88°, was recorded at a location 9½ feet from the floor, 8¼ feet from the eastern alleyway, and slightly to the north of the center of the mow. On September 5 several thermocouples still registered as high as 70°.

EXPERIMENT 4, JUNE 1931

In experiment 4 the hay was stored on June 2 to 4. Dry timothy containing 10.10-percent moisture was put in up to a height of 4½ feet from the floor; 14,900 pounds of undercured alfalfa, having an average moisture content of 56.10 percent, was put over an area of 10 by 10 feet in a central section of the mow, up to a height of 12½ feet; the undercured section was covered and surrounded to a height of 16½ feet by cured alfalfa having an average moisture content of 28.46 percent. The maximum temperature, 78° C., was recorded on June 15 at thermocouple 25, in the cured alfalfa just above the center of the undercured section. The temperature here had dropped to 71° on June 26, on which date the three next highest temperatures were 68°, 63.5°, and 61.5°. When the hay was removed from the barn, on July 6 to 8, the settled height was 10 feet.

EXPERIMENT 5, JULY 1931

The hay used in experiment 5 was dry timothy, containing 16.08-percent moisture, on the bottom of the mow to a height of 2 feet; cured alfalfa of 19.78-percent moisture content to a height of 6½ feet; undercured alfalfa of 31.34-percent moisture content to a height of 9½ feet; and dry timothy on top of this to a height of 15 feet. The maximum temperature, 56° C., was reached on July 19 to 20, 6 days after storage (July 13 to 16), at thermocouple 18, located at the junction of the cured and undercured alfalfa in the southeast section of the mow. On August 20, when the hay was removed from the barn, the maximum temperature was only 35.5°.

EXPERIMENT 9, JUNE 1933

The hay in experiment 9 was stored from June 5 to 13 and removed on October 10. It consisted of cured alfalfa, containing 29.86-percent moisture, on the bottom of the mow to a height of 4½ feet; undercured alfalfa of 43.17-percent moisture content to a height of 8½ feet over an area of 10 by 10 feet in a central section; and cured alfalfa of 29.86-percent moisture content surrounding and covering the undercured hay to a height of 16½ feet. At the time of removal the hay had settled to a height of 13 feet. The highest temperature, 68° C., was reached on June 13 at thermocouple 20, located 6 feet from the floor, at the exact center of the junction of the cured and undercured hay. From June 14 the temperature throughout the mow fell rapidly until July 5, when thermocouple 20 registered 60°.

APPARATUS FOR GAS SAMPLING

The apparatus used in the collection of the samples of gas is shown in figures 1 and 2. The gas-sampling device (fig. 1; A, B, and C), consists essentially of an aluminum capillary tube (*a*) having a bore

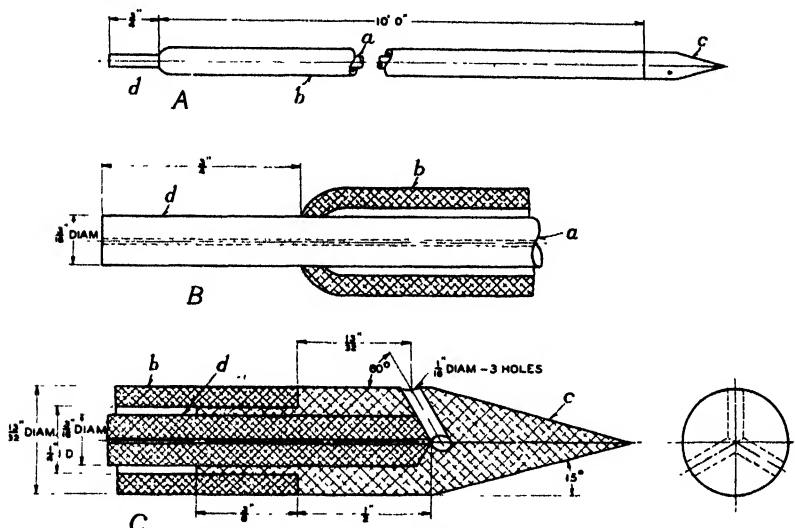


FIGURE 1.—Gas-sampling device: A, complete device; B, end of tube opposite removable tip; C, removable tip.

of approximately 0.02 inch supported by a larger tube (*b*) of the same metal of 13/32-inch diameter. It is through this capillary tube that the sample of gas passes during collection. The length of tube used

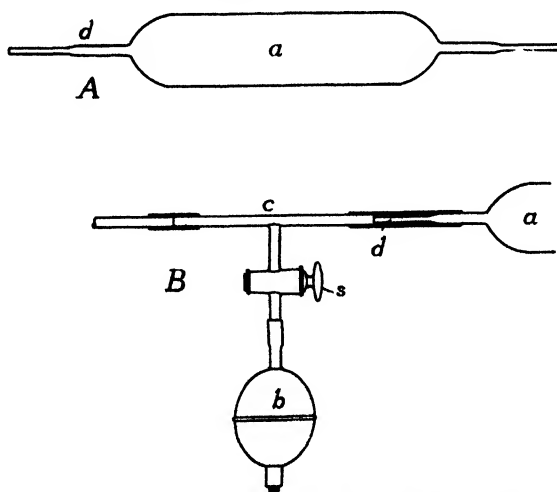


FIGURE 2.—Gas-sampling tube and connections: A, tube; B, tube and connections.

is determined in each case by the distance to which it is desired to penetrate the mow for sampling. Five-, ten-, and twelve-foot lengths have been found satisfactory. In operation, the end of the tube

carrying the removable tip (*c*) is inserted to the desired point in the mow and the other end of the tube (*d*) is connected by rubber tubing through the capillary T-tube (fig. 2, *c*) to one end of the gas-sampling tube (fig. 2, *a*). The glass tube (*a*) has a capacity of approximately 250 cc. Previous to use it is sealed off at one end, highly evacuated, and then sealed off at the other end. The metal capillary and glass connections are then cleared of air by closing the stopcock (*s*), compressing the rubber bulb (*b*) attached to the third arm of the capillary T-tube, and then reopening the stopcock, allowing gas to be drawn into the bulb. Then the stopcock is closed again, and the scratched tip of the gas-sampling tube is broken at (*d*), whereupon the desired sample of gas is drawn by vacuum into the gas-sampling tube. Immediately upon detaching, the broken tip of the gas container is inserted airtight into a wax-sealing material contained in a small metal tube, such as a .32-caliber cartridge. This material is a mixture of beeswax and Venice turpentine in proportions varying with the temperature at which it is to be used. The sample is then ready for analysis. A Burrell precision-model gas-analysis apparatus is admirably adapted for this purpose.

RESULTS

The results of the analysis of the gases collected in the several experiments, together with other data pertaining to the samples, are given in table 1.

TABLE 1.—Analyses of gas samples collected in large-scale spontaneous heating experiments

EXPERIMENT 1, JUNE 1929¹

Sample no.	Date of sam- pling	Period after storage of hay	Location in mow					Nearest thermo- couple	Temper- ature at nearest thermo- couple on day of sam- pling	Previous maximum temperature at same locality		CO ₂ con- tent	O ₂ con- tent	O ₂ loss (20.88- O ₂)	Ratio CO ₂ to O ₂ loss
			Height from floor	Distance from north	Distance from south	Distance from east	Distance from west			Temper- ature	Date				
1	June 10	3	9½	8	8	8	8	29	50.5	45.5	June 9	0.10	20.69	0.17	
2	do	3	9	0	16	14	2	25	53.0	63.0	do				
3	June 13	6	9	1½	14½	11½	4½	22	50.5	53.0	June 8	.12	20.46	.40	
4	do	6	8	11	5	8½	7½	26	70.0	71.0	June 12	1.77	18.74	2.12	0.835
5	June 21	14	10	3	13	6	10	24	63.5	67.0	June 12	4.18	15.87	4.99	.838
6	do	14	9	8	8	8	8	22	57.0	71.0	do	2.68	17.68	3.18	.843
7	June 28	21	5	8	8	7½	8½	29	51.0	61.5	June 11	2.42	18.28	2.88	.938
8	do	21	5	8	8	9½	7½	25	60.5	70.5	June 15	3.94	16.87	3.99	.987
9	Aug. 27	81	5	8	8	7½	8½	17½	60.0	72.0	do	3.80	16.90	3.96	.960
10								17½	49.0	72.0	do	1.45	19.34	1.52	.954

EXPERIMENT 3, JULY 1930

1	July 28	5	2½	11½	11½	11	11	0	82.0	79.0	July 27	1.32	19.42	1.44	0.916
2	do	5	5	11½	11½	5½	16½	20	86.0	81.0	do	13.72	5.75	13.11	.908
3	do	5	5	11½	11½	3½	18½	28	82.0	82.0	do	13.46	5.93	14.01	.903
4	do	4	6½	17½	5½	5½	16½	28	76.0	82.0	do	7.53	12.54	8.32	.895
5	Aug. 5	13	2	11½	11½	5½	16½	28	77.0	82.0	do	10.25	6.31	12.35	.895
6	do	12	6½	17½	5½	5½	16½	28	84.0	82.0	July 28	5.26	14.22	6.84	.867
7	Aug. 14	21	7½	6½	16½	6½	15½	32	84.0	83.0	Aug. 1	16.60	3.94	17.92	.897
8	do	21	7½	7	16	7	15	32	69.0	81.0	do	5.21	14.95	5.81	.887
9	Sept. 9	47	5½	11½	11½	11	11	32	69.0	81.0	Aug. 24	11.40	9.43	11.53	.894
10	do	47	4½	11½	11½	11	11	32	69.0	81.0	do	13.34	7.31	13.85	.894

¹ Sample 1 was taken 1 inch below top of mow. Samples 2, 3, and 4 were taken from hot spots, 1, 1½, and 2 feet below top of mow. Samples 5 and 6 were collected 3 and 4 inches from top of mow.

² Above top of stack.

TABLE 1.—Analyses of gas samples collected in large-scale spontaneous heating experiments—Continued

EXPERIMENT 4, JUNE 1931²

Sample no.	Date of sam- pling	Period after storage of hay	Location in row					Nearest thermo- couple	Temper- ature at nearest thermo- couple on day of sam- pling	Previous maximum temperature at same locality		CO ₂ con- tent	O ₂ con- tent	O ₂ loss (20.96— O ₂)	Ratio CO ₂ to O ₂ loss
			Height from floor	Distance from north	Distance from south	Distance from east	Distance from west			Temper- ature	Date				
												° C.	° C.		
	June 24	Days	Feet	Feet	Feet	Feet	Feet	25	72.5	78.0	Percent	Percent	Percent	0.87	
	do.	19	9	13	11	11	12	26	56.0	61.0	18.33	18.33	2.53	1.01	
	do.	19	10	10½	13½	17	6	27	72.5	78.0	1.87	19.01	1.85	80	
	do.	19	8	12	12	9	14	20	72.5	78.0	2.63	17.90	2.96	1.02	
	do.	19	7	14½	9½	13	10	20	71.5	73.5	2.21	18.60	2.17		

EXPERIMENT 5, JULY 1931³

1	Aug. 17	32	4½	18	6	6½	16½	18	37.0	July 19	0.66	20.20	0.66	1.00
2	do.	32	6½	12	12	9	14	14	32.0	July 20	.59	20.48	.38	1.56
3	do.	32	7	12	12	11½	11½	25	31.0	do.	.43	20.77	.09	
4	do.	32	7	16	8	7	16	20	30.0	do.	.78	20.57	.29	2.70
								18	31.0	July 19				
								11	36.5	July 18				

EXPERIMENT 9, JUNE 1933⁴

1	June 21	16	6	13	11	10	13	20	66.5	June 13	3.20	18.78	2.06	1.54
2	do.	15	4	13	11	10	13	14 ^y	68.0	June 10	2.96	18.77	2.09	1.42
3	do.	15	4	13	11	10	14½	14	63.0	June 13	2.30	19.43	1.43	1.61
4	July 7	31	5	13	11	10	13	20	56.5	do.	3.96	17.91	2.95	1.34
5	do.	31	4	13	11	8½	14½	14 ^y	54.0	June 10	3.11	18.82	2.04	1.52
6	July 19	43	5	13	11	10	13	20	56.0	do.	3.02	17.98	2.88	1.06
7	Aug. 3	58	4	13	11	8½	14½	14 ^y	51.5	June 13	.91	20.20	.66	1.38
8	do.	58	5	13	11	10	13	20	53.0	do.	2.16	19.07	1.70	1.21
								y	53.5	June 10				

² Samples 1, 2, and 3 were taken from areas of cured alfalfa which had become wet from use of H₂O, from undercured section below. Sample 4 was taken from undercured section.

³ Sample 1 was collected from junction of cured and undercured alfalfa; sample 2, from center of undercured alfalfa; sample 3, from cured alfalfa; sample 4, from undercured alfalfa.

⁴ Sample 1 was collected at the junction of cured and undercured alfalfa; all other samples were from the undercured section.

All the samples in experiments 1, 3, 4, and 5 were analyzed for carbon monoxide, hydrogen, and methane. Carbon monoxide and hydrogen were determined by combustion in the copper oxide tube at 290° to 310° C. (after removal of constituents soluble in caustic potash, fuming sulphuric acid, and pyrogallol), and methane was determined in the slow-combustion pipette. Methane was absent in all the samples, and neither carbon monoxide nor hydrogen was found in the samples taken in experiments 4 and 5. Carbon monoxide and hydrogen were found in only one sample in experiment 3, and the amounts in that were within the limits of experimental error. One sample in experiment 1 appeared to contain approximately 0.1 to 0.2 percent of carbon monoxide, while the contraction of the gas upon combustion over copper oxide in five of these samples indicated a possible hydrogen content of 0.1 to 0.3 percent. In several of the samples in experiments 1 and 3, a considerable quantity of the gas was removable by fuming sulphuric acid. Tests for phosphine and hydrogen sulphide on all samples in experiment 1 and on a large proportion of those in experiment 2 gave negative results. Practically all the samples of gas had a peculiar pungent odor similar to that of aldehydes and esters.

It will be observed that in experiment 1 the samples of gas from sources where temperatures of 68° to 70° C. prevailed at the time of collection, or previously, show an oxygen loss greater than the carbon dioxide formed.

The data for experiment 3 also show definite evidence of excess oxygen consumption. Furthermore, the large quantities of carbon dioxide formed in the heating haymow, combined with the high degree of exhaustion of the available oxygen shown by most of the samples, indicates more than ordinary activity. This indication is borne out by observations made of the physical qualities and appearances of the hay when removed from the mow, namely the preponderance of spoiled, brown and black, acid-charred material.

The activity in experiment 4, where a maximum temperature of 78° C. was observed, is fairly comparable with that in experiment 1, with a maximum of 72°, notwithstanding the fact that the absolute quantities of carbon dioxide in the samples are generally somewhat lower. The conditions of storage and the smaller volume and weight of the wet hay may have permitted a greater dilution of the gases with air from outside sources, which, in spite of the slightly higher and longer prevailing temperatures, would cause a lower carbon dioxide content and a correspondingly lower value for the oxygen consumption.

In experiment 5, the relatively small volume of undercured hay and its comparatively low moisture content appear to account for the low degree of activity, with low carbon dioxide production and lower oxygen loss. The results as a whole may be taken to indicate conditions of the ordinary "sweating" process.

In experiment 9, 68° C. was the maximum temperature observed, and it will be noted that the $\text{CO}_2:\text{O}_2$ -loss ratio is greater than unity in all the samples.

LABORATORY OXIDATION EXPERIMENTS

PROCEDURE

The purpose of this laboratory investigation was to determine the relative tendency to oxidation of natural undecomposed alfalfa hay and similar alfalfa hay which had undergone spontaneous heating in the mow or had been fermented upon exposure while wet. It was premised that if unsaturated or other easily oxidized substances were present in the decomposed samples, these samples should absorb relatively more oxygen than the undecomposed samples, and would throw light on the question of the production of unsaturated oxidizable substances during spontaneous heating. The difficulty of securing samples of heated hay from the mow without exposing them to the air was recognized at once; hence, in the first large-scale experiment, nitrogen was constantly passed through the boring tube used for removing the samples and the sample containers were filled in an airtight box in which the air had been displaced by nitrogen. Analysis of the protecting gas taken from the sample containers (half-gallon fruit jars) at the time the samples were removed for oxidation tests proved the efficiency of this method. As these samples did not show much absorption of oxygen, the use of protecting gas was abandoned in subsequent experiments but every other precaution was taken to avoid undue exposure.

A temperature of 80° C. was selected for the comparison because of the general agreement among investigators that at this temperature all microbial activity has ceased. To confirm the presumption that such action is absent also in the hour's time of attaining the excluding temperature range, this initial heating was investigated. Experiments were conducted at 90° to show the effect of higher temperature. The possible analogy of such oxidations to the well-established avidity of linseed oil and other unsaturated oils to take up oxygen was not overlooked, and a test on boiled linseed oil impregnating an equal weight of cotton waste was conducted under identical conditions.

APPARATUS

The apparatus in which these experiments were conducted is shown in figure 3. *a* is a Pyrex round-bottom flask having a capacity of 1,054 cc when tightly closed with the three-hole rubber stopper *e* carrying a thermometer and a capillary tube which emerges through the stopper to *f*. A small condenser tube (internal diameter about 4 mm) of the condenser *d* is connected through the third hole of the stopper. This tube is joined, by means of rubber tubing at *g*, to a capillary tube bent as shown, which in turn is connected at *h* to the small end of the inverted separatory funnel *b* of approximately 500-cc capacity joined at the tubulature by rubber tubing to a leveling bulb. Above the point *h* a capillary tube is sealed on at a right angle and connected with a small open mercury manometer *m*. On the other side of the flask *a*, connection is made from *f* by capillary tubing to *k*, where it is joined by rubber tubing to *c*, a cylindrical glass tube having a capacity of about 300 cc, and provided with stopcock *l*. This container also is provided with a leveling bulb. *o* is a cylindrical oil bath in which flask *a* is immersed in heavy mineral oil. This bath is heated from below by means of an electric hot plate *p*, and within the

oil by an electric heater *n*, carrying a stirrer and regulated by means of a bimetallic thermostat *r*. The oil bath has an inside diameter of 33 cm and is well insulated on its sides with a 5-cm thickness of asbestos. By means of the regulator and heater, temperatures can be maintained to within 0.4°C . The total volume of the closed system from stopcock *s* to stopcock *l*, including flask *a*, the condenser, manometer tube, and capillary tubing of 0.5-mm diameter, is 1,060 cc.

In conducting an experiment, the mercury used in *b* and *c* is raised to stopcocks *s* and *l*, respectively, and the stopcocks are closed. A weighed sample is then placed in *a*, the rubber stopper carrying the thermometer, the condenser, and the capillary tube is tightly inserted, and connections are made at *f* and *g* by means of rubber tubing. The temperature of the air and sample and the barometric pressure are noted at the time of closing the system, and the enclosed air volume (1,060 cc) is later corrected for air displaced by the sample. This displacement is calculated from the specific gravity determined experimentally on the dry matter contained in several representative types

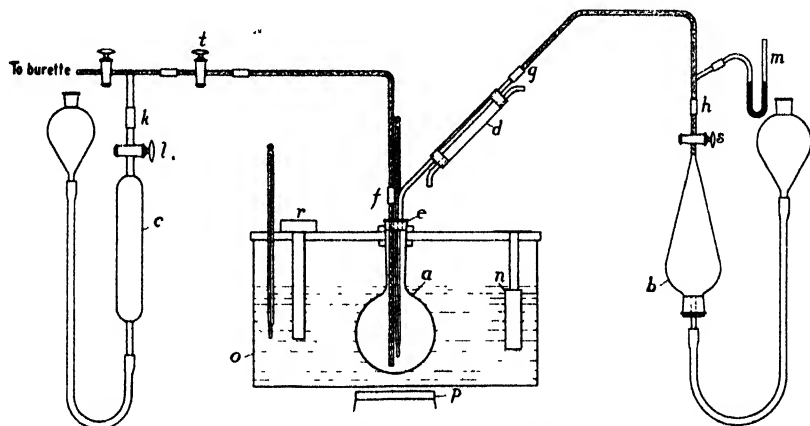


FIGURE 3.—Apparatus for oxidation experiments.

of samples. From the average of these figures, which varied little, a correction value was obtained of 0.7 cc of air displaced per gram of dry matter. This corrected volume is reduced to the arbitrary standard conditions of 25°C and 760 mm pressure, the tension of water vapor being taken into account.

The bath is now heated by the electric hot plate until the temperature has reached to within about 10° of the desired bath temperature; then the heater within the oil is substituted as the source of heat and serves for all subsequent heating. As the temperature of the flask *a* and its contents rises, the excess of expanding air is allowed to collect in container *b*, the stopcock of which has been opened at the start of heating. During this time and throughout the entire experiment, the level of the mercury is kept adjusted to atmospheric pressure as indicated by the manometer levels. If, as in some experiments, container *b* is not sufficient to take care of the air expansion, container *c* also may be used for the purpose. The small condenser *d* serves to condense and avoid loss of moisture in the sample.

When the bath reaches the temperature desired, the temperature in flask *a* containing the hay sample is recorded as the initial temperature for the subsequent 4-hour period of heating at the fixed bath temperature. Temperature readings are recorded at frequent intervals during the heating. The temperature of the sample and air in the flask was found to be always somewhat lower than the bath temperature, in most cases probably owing to the evaporation of water.

At the end of the 4-hour period the bath is cooled as rapidly as possible by means of cold water passed through cooling coils located around the interior walls of the bath. During the cooling some of the previously expanded air collected in *b* is returned to *a* to take care of the contraction in *a*. When the bath temperature has fallen to 50° to 60° C., stopcocks *t* and *l* are opened, and the gas is passed back and forth through *a* from *b* to *c* until the entire volume of gas has become thoroughly mixed. Finally all the gas is removed from *b*, and stopcock *s* is closed. The sampling of gas for analysis is then completed by drawing into *c* the volume required for several analyses. From *c* the gas can be transferred readily through capillary connection into the burette used in the analysis.

The time required to raise the oil bath from room temperature to 80° C. was remarkably uniform in the experiments, the records showing a maximum of 68, a minimum of 52, and an average of 61 minutes. To raise it to 90° required only a few minutes longer. The cooling from 80° to 90° to 50° to 60° required an average of 23 minutes.

RESULTS

The results of the laboratory experiments are recorded in table 2.

TABLE 2.—Results of laboratory experiments on the oxidation of hay

UNDERCURED ALFALFA AND SLIGHTLY FERMENTED ALFALFA (BATH TEMPERATURE, 80° C.)

Sample no. and condition	Weight of sample Grams	Moisture content		Dry matter		Temperature of sample °C.	Volume of original air (mm) (79.14 percent N ₂) (A)	Composition of original air (B)		Volume final gas based on N ₂ (C)	Composition of final gas (D)						Loss of gas ¹ (A) - (C)	Available O ₂ at maximum temperature (E)	O ₂ absorbed (B) - (D)	CO ₂ formed	O ₂ absorbed per 25 g ash-free dry matter	CO ₂ formed per 25 g ash-free dry matter	Ratio CO ₂ formed to O ₂ absorbed
		Grams	Pct.	Weight	Ash			O ₂	N ₂		CO ₂		H ₂										
											Pct.	Cc	Pct.	Cc	Pct.	Cc							
3(8)	50	46.30	28.53	9.97	75.0-77.4	1,010	60.210	81.799	999.86	3.46	34.59	16.55	165.48	79.99	799.79	10.74	107.49	45.33	34.59	45.37	34.62	0.763	
4(9)	40	33.90	23.64	7.78	74.0-77.0	971	18.202	59.768	993.57	4.82	36.54	15.98	150.44	79.60	768.59	5.61	107.27	62.15	46.54	49.21	49.21	0.892	
5(9) fermented	40	39.00	24.70	7.42	78.0-78.5	1,002	30.208	68.793	982.45	3.72	36.55	15.54	152.67	80.74	793.23	19.55	103.05	56.41	36.55	62.87	40.51	0.648	
6(9) moldy	40	38.26	24.70	7.78	74.0-77.0	1,031	45.215	16.816	926.52	5.10	52.35	15.38	157.88	79.52	816.29	4.83	103.06	56.41	52.35	62.87	57.46	0.914	
7(10)	40	37.10	25.16	7.78	77.0-77.6	990	52.206	62.783	987.03	5.83	57.54	14.75	145.59	79.42	783.90	3.49	105.65	61.03	57.54	65.94	61.99	0.943	
8(10)	50	48.42	25.79	9.89	76.0-77.6	1,011	82.211	66.900	995.10	4.48	44.58	15.05	149.76	80.07	800.76	16.72	105.65	61.30	44.58	65.94	47.95	0.727	
9(4)	50	56.16	21.93	9.89	75.0-77.0	957	28.195	68.757	946.88	4.55	43.08	15.44	146.20	80.01	757.60	10.40	105.21	63.48	43.08	67.66	54.50	0.806	
10(4)	60	69.80	18.12	9.89	75.0-77.5	998	50.208	29.700	988.28	4.53	44.77	15.51	153.28	79.96	790.21	10.24	97.29	55.01	44.77	84.22	68.55	0.814	
11(4) fermented	50	57.78	21.11	9.89	75.0-77.5	964	63.201	22.763	946.10	6.45	61.02	12.96	121.67	80.69	763.41	18.53	104.78	79.55	61.02	104.55	80.19	0.767	

UNDERCURED ALFALFA AFTER SPONTANEOUS HEATING (BATH TEMPERATURE, 80° C.)

40	A(9)	31.28	10	75.4-76.0	1,032	20	215.32	816.98	1,031.41	0.72	7.42	20.08	207.11	79.20	816.88	0.79	111.57	8.21	7.42	7.29	4.59	0.604
40	B(10)	30.78	9.08	74.0-77.1	1,015	27	211.79	803.48	1,023.29	1.74	17.81	19.74	202.00	78.52	803.48	+8.02	101.25	9.79	17.81	8.74	15.91	1.819
30	B(24)	24.30	14	71.2-73.6	1,026	69	214.16	812.53	1,026.16	0.72	7.39	20.10	206.26	78.18	812.53	-5.11	119.21	7.90	7.39	9.45	8.84	1.355
40	B(30)	30.20	10	73.0-77.0	1,005	38	209.72	795.06	1,006.10	1.78	17.97	19.42	196.09	78.80	795.06	-4.34	106.49	13.63	17.97	12.50	16.49	1.318
40	B(31)	30.20	11	71.74-0-77.0	1,014	71	211.69	803.08	1,006.12	2.02	20.61	19.26	196.48	78.62	803.08	+5.40	107.12	15.21	20.61	14.55	19.45	1.355
40	B(64) ²	23.47	12	73.6-75.0	1,027	14	214.21	812.88	1,027.65	1.35	13.87	19.55	200.91	79.10	812.88	+5.52	115.35	13.35	13.87	16.78	16.78	1.039
40	B(26)	24.08	11	99.75-0-77.0	1,037	12	216.34	920.78	1,034.64	1.65	17.07	19.02	196.79	79.33	920.78	2.48	107.95	19.35	17.07	23.07	20.13	0.873
40	B(84) ²	28.03	15.40	76.2-78.8	1,002	22	209.06	793.16	959.89	6.65	63.83	10.72	102.90	82.62	793.16	42.33	101.55	106.16	63.83	111.91	67.29	0.601

¹ Increase of gas volume is indicated by plus (+) sign.

² Analyzed for combustible constituents.

In this table the samples of hay are designated by a combination of numbers or of letters and numbers. The letter S indicates that the sample was the standard laboratory sample drawn before storage and corresponding to the hay stored in wire-cloth containers (or "baskets") in the respective large-scale experiments. The letter B indicates that the sample was from one of such baskets after storage. The number in parentheses is the number of the large-scale experiment. In the sixth column, which shows the temperature range during the 4-hour heating period, the initial temperature of the period is given as the lower limit, but the temperature prevailing during practically all that period was very close to the maximum temperature recorded. For the purpose of more accurate comparison the oxygen absorbed and carbon dioxide formed are computed for equal amounts (25 g) of moisture and ash-free sample, and with the exception of section 4 the results are arranged in the order of increasing oxygen absorption. Available O_2 at maximum temperature is that portion of the original oxygen remaining in the oxidation flask when the air had been expanded by heating to the maximum temperature of the experiment. All volumes given in the table are for $25^\circ C.$ and 760 mm.

The values given in the table for oxygen absorbed and carbon dioxide formed are not corrected for the relative solubility of these gases in the water of the hay samples at the temperature and pressure of sampling. Such corrections would not exceed 1 cc for carbon dioxide and 0.15 cc for oxygen in the hay sample with the highest water content, and in no case would the tabulated results be materially affected by these corrections.

Some of the samples of gas were analyzed for combustible constituents, with negative results.

In the case of boiled linseed oil, however, the combustion over copper oxide resulted in a contraction of volume equivalent to 0.16 percent of hydrogen and a production of carbon dioxide equal to 0.32 percent of carbon monoxide. Attention is called to a recent experiment by Haldane and Makgill (5) in which carbon monoxide occurred in the gas resulting from the oxidation of boiled linseed oil at $18^\circ C.$ According to Haldane and Makgill, small quantities of carbon monoxide are formed also in the oxidation of wet hay at 40° , when bacterial activity is entirely excluded. Under the influence of bacteria, however, hydrogen is formed.

In table 2, section 1, S(8) was a sample of undercured alfalfa taken in September 1932 from a field in the vicinity of Beltsville, Md. Sample S(9) was undercured alfalfa from another field near Beltsville, collected in June 1933. Sample S(9) fermented was a sample of the same hay after exposure in the laboratory to warm moist air for 3 weeks, while S(9) moldy had been exposed to room temperature for 11 days in a loosely closed jar. Sample 1(10) was undercured alfalfa collected on June 12, 1934, from a field also in the vicinity of Beltsville. S(4), 7(4), 9(4), and 6(4) were undercured alfalfa from the dairy husbandry farm at Beltsville. Sample 6(4) was slightly sour when tested.

The effect of moisture is indicated in samples 7(4), 9(4), and S(4) of hay from the same field. The oxidation increases with the moisture content. In samples S(9) fermented, which had undergone microbial changes, and S(9) moldy, in which considerable mold had formed, the oxidation was greater than in S(9). For apparently the same reason

the oxidation was greater in the soured sample 6(4) than in samples 7(4), 9(4), or S(4). The ratio of CO₂ formed to oxygen absorbed is less than unity in all experiments.

In table 2, section 2, samples A(9) and B3(9) were from the same hay as S(9) (sec. 1) but had undergone spontaneous heating. These samples were collected from a location in the mow (table 1, experiment 9) where a maximum temperature of 68° C. was reached 7 days after storage and where a temperature between 68° and 60° prevailed for 28 days afterward. Sample B3(9) had undergone a decomposition loss in dry organic matter of nearly 14 percent. Samples 18(1) and 13(1) were similar, having been collected in experiment 1 from localities in which an average temperature of 68° had prevailed for several days. Samples B2(4), B6(4), 25(4), and B8(4) were the result of the heating during storage of undercured hay S(4), 7(4), and 9(4) (sec. 1). Samples B2(4) and B6(4) had been stored in a section of cured alfalfa where the temperature for about 25 days averaged 55° and 58°, respectively; B2(4) had lost 22 percent and B6(4) 19 percent of dry organic matter. Sample 25(4) was collected from a location in the originally undercured alfalfa where the temperature had ranged from 68.5° to 60° for 30 days. Sample B8(4) had undergone a heating of 73.5° to 63° for 26 days in a wire basket located in the undercured alfalfa section of the mow. This sample had lost a little less than 11 percent of dry organic matter during storage, but the metal basket had become badly corroded, and the sample was contaminated with copper and nickel as indicated by the high ash content and proved by the analysis of the ash.

With the exception of this contaminated sample the results for all the experiments in section 2 show very low oxygen absorption and carbon dioxide formation as compared with those in section 1. It is possible that this is due largely to the comparatively lower moisture content of the samples in section 2, although such an effect is not indicated in sample 25(4), which has the highest moisture content but only a slightly greater oxygen absorption and carbon-dioxide formation. Apparently the lower results must be ascribed to the losses of organic matter during storage and to the previous destruction of any unsaturated substances that might have been formed in the course of spontaneous heating.

The experiments recorded in section 3 of table 2 were carried out at temperatures about 10° higher than those in sections 1 and 2. The effect of this higher temperature is shown in the increased oxidation in 5 of the 6 cases, excluding sample B6(4), to which water had been added, and in an increase in the proportion of carbon dioxide formed to oxygen absorbed in 4 of the same 6 cases. In sample B8(4) the oxygen available for oxidation at the maximum temperature had been greatly decreased from the corresponding value shown in section 2 by the expansion accompanying the elevation of temperature.

The experiment with sample S(8) (sec. 4) was designed to show the behavior of undercured alfalfa when subjected to oxidation under conditions believed to be favorable for microbial action. Accordingly a sample was kept at a temperature of 45° C. for 78 hours in the same apparatus and according to the identical procedure followed in the other experiments at higher temperatures. In this experiment the oxygen absorbed was greater, but the carbon dioxide formed was less

than in the experiment with the same hay at 75° to 77.4° (sec. 1). The ratio of CO₂ formed to O₂ absorbed was consequently lower than at the higher temperature.

The alfalfa of previous experiments, sample S(4), completely dried and ground, was heated as shown in table 2, section 4. There was very little oxidation, but the carbon dioxide exceeded the oxygen loss. To another sample of the same dry and ground hay an equal weight of water was added, and the oxidation was observed. The effect of this wetting is quite apparent in the enormously increased oxidation. Here again the carbon dioxide formed exceeded the oxygen absorbed.

A third sample of this dry, ground hay heated around 100° C. showed a greater oxidation than the same dry hay at 85.5° to 90.5°, with a reversal of the ratio of carbon dioxide formed to oxygen consumed. Presumably some destructive distillation was to be expected here, and if it occurred it appears to have been accompanied by the formation of products that increased the relative absorption of oxygen.

The results of the experiment with boiled linseed oil show the character and comparative degree of the oxidation of unsaturated oil and very probably furnish the clue to the nature of the chemical oxidations of hay, insofar as these may involve the oxidation of unsaturated substances formed as the result of heating.

The character and extent of the oxidation which occurs in the interval of approximately 1 hour required to bring the temperature up to the temperature at which the 4-hour period of heating begins are shown in the case of samples 7(4)a and 1(10)a. It is evident that this initial oxidation is considerable, but of essentially the same character as shown by the other experiments with the same hay, in which the heating was continued for the usual 4 hours, samples 7(4)b and 1(10)b. Furthermore, if the values for oxygen consumed and carbon dioxide formed in samples 7(4)a and 1(10)a are subtracted from the corresponding values for samples 7(4)b and 1(10)b, it will be seen that the ratios of carbon dioxide formed to oxygen consumed in the 4 hours in which the oxidation was confined to the maximum temperature are not materially affected.

Experiments were made to determine the effect of heating more than the arbitrary 4 hours. Sample 1(10)c was heated for 20 hours. It was then heated for an additional 20 hours with a fresh supply of air, sample 1(10)d. The results show a progressive oxidation, incomplete but of approximately the same character.

DISCUSSION

The results of the laboratory oxidation experiments (table 2) considered in relation to the results of the analysis of the gases collected in the large-scale experiments (table 1) throw considerable light on the question of chemical activity in the heating haymow. In the first place, the laboratory experiments at temperatures between 77° and 80° C., where chemical oxidation occurred in natural undercured alfalfa without the intervention of micro-organisms, show that at these temperatures chemical action is accompanied by an oxygen consumption in excess of the carbon dioxide formed, and that this oxidation is progressive and of the same character regardless of whether it is limited to 4, 20, or 40 hours (table 2, secs. 1 and 4).

On the other hand, the experiments with undercured hay which had already suffered oxidation in the mow show that at these same temperatures chemical oxidation may result in the production of carbon dioxide in excess of the oxygen consumption (table 2, sec. 2). Furthermore, the results of heating at approximately 90° C. show that at this temperature there is increased chemical activity accompanied by an increase in the proportion of carbon dioxide formed to the oxygen absorbed (table 2, sec. 3). It is very probable that in these samples some of the oxygen combined chemically in the earlier stages of chemical oxidation may be liberated as CO₂ in the later stages, in which case the carbon dioxide produced may exceed the oxygen absorbed.

It may be concluded, therefore, in harmony with the previous conclusions of Haldane and Makgill (4), that a consumption of oxygen greater than the liberation of carbon dioxide is an evidence of chemical oxidation. But it does not follow that all chemical activity is limited to those instances in which the carbon dioxide liberated is less than the oxygen consumed.

If, therefore, an oxygen consumption in excess of carbon dioxide production is evidence of chemical oxidation, in order to determine the occurrence of chemical action in the presence of biological activity in the spontaneous heating of hay, it is necessary to assume that the production of carbon dioxide in amounts equal to or greater than the oxygen consumed is characteristic of biological action. This assumption appears to be justified by the observations of Haldane and Makgill referred to above, and it is in agreement with the results of extensive investigations of the mechanism of plant respiration and micro-organic activity, at least where the resultant gaseous product is carbon dioxide alone.

Upon the basis of this distinction between chemical and biological activity, the results of the investigation of the gases collected in the large-scale spontaneous-heating experiments show that, along with the respiration processes of the living plant cell and the activity of micro-organisms, chemical oxidation occurred where a temperature range of 68° to 70° C. prevailed (table 1, experiment 1). For the production of the temperature required for the initiation of this chemical action, in the absence of other evidence, the operation of biological agencies must be assumed. Upon the same basis, chemical activity is established also for temperatures between 72° and 78° (experiment 4), and this is still more positively indicated in experiment 3, where temperatures between 76° and 88° prevailed for several weeks.

The results of experiments 5 (maximum temperature, 56° C.) and 9 (maximum temperature, 68°) do not show chemical oxidation, but they do not exclude the possibility of such oxidation as may have occurred along with the greater activity of micro-organisms.

From the evidence thus far presented the conclusion is justified that chemical oxidations take place in the heating haymow at a temperature as low as 68° C. The evidence of chemical activity is not so satisfactory for temperatures much below this. However, the laboratory experiment on sample S(8) (table 2, sec. 4) may be interpreted as showing chemical oxidation combined with microbial action. As already stated, this experiment was expected to show the behavior of undercured alfalfa subjected to oxidation under

conditions believed to be favorable for microbial action. In this experiment the hay was kept at 45° for 78 hours, and the same procedure was followed as in the other experiments at higher temperatures. Here the relation of carbon dioxide formation and oxygen consumption was found to be of the same order and character as in the same alfalfa at approximately 77°. The $\text{CO}_2:\text{O}_2$ loss ratio was far below unity. If this experiment may be accepted as showing chemical action at 45°, it would be reasonable to conclude that, throughout the whole range of temperature usually ascribed to the action of biological agencies, chemical oxidations also occur, simultaneously or alternately. The results obtained in the investigation of the oxidation occurring in the interval of approximately 1 hour required to raise the temperature of the hay samples to approximately 75° (samples 7(4)a and 1(10)a, table 2, sec. 4) lend support to this conclusion. This initial oxidation was considerable and of essentially the same character as the subsequent oxidation up to a maximum of approximately 80°.

The existence of chemical activity in a mass of hay in which the temperature has risen from any cause whatever to the neighborhood of 80° C. is further confirmed by the fact that in the laboratory experiments such activity was produced by heat supplied from external sources. Similar activity obviously must be inferred in the case of the heating mass of hay at a corresponding temperature.

The discussion of the results of this investigation would not be complete without a consideration of the significance of an oxygen consumption in excess of the carbon dioxide liberated in the laboratory oxidation experiments. Perhaps the occurrence of small quantities of unsaturated substances in the hay may have some influence. Again, it might be assumed that there is an unequal absorption or adsorption by the heated hay of oxygen and carbon dioxide, which would affect the ratio of carbon dioxide formed to oxygen consumed. But under the conditions of experimentation it does not appear at all probable that the hay was converted into such a condition that it would have much adsorptive property. It is far more probable that the excess oxygen consumption was mainly owing to incomplete oxidations in which there was no corresponding carbon-dioxide formation, or to the production, under the influence of heat and chemical action, of unsaturated substances which were destroyed at once by oxidation.

Unfortunately, because of the nature of the experiments, it was not possible to obtain direct proof of the presence of unsaturated substances in the products of reaction. The same difficulty was experienced in the effort to show their presence in the samples of undercured hay which had undergone spontaneous heating in the mow prior to the laboratory tests (table 2, sec. 2). The comparatively low oxidation results in the case of these samples indicate that a partial or complete oxidation of any easily oxidized unsaturated products that might have been formed there had already occurred in the mow.

The desirability of further investigations to determine whether or not unsaturated substances are readily formed in hay, either by purely chemical processes or by the action of micro-organisms, is quite obvious. And if such investigations should demonstrate that under the influence of heat supplied from external sources, with the exclusion

of microbial activity, unsaturated products are formed, the interesting question would arise whether chemical activity in the heating hay-mow may not be due in part to the production by heat of unsaturated substances susceptible to oxidation. However, it would be necessary to assume that the heat production in the mow is due mainly to biological agencies, and consequently the distinction between the formation of unsaturated substances by microbial agencies and their formation as the result of the heat of microbial activity would be one only of proximate or ultimate cause.

SUMMARY

The results of an investigation of the gases collected during the spontaneous heating of alfalfa hay and comparative results of oxidation experiments in the laboratory are presented.

Apparatus for the collection of gases from the heating haymow and a laboratory oxidation apparatus are described.

The results of the investigation indicate that, along with the operation of biological agencies in the heating haymow, there occurs a purely chemical oxidation, evidenced by a loss of oxygen considerably in excess of the carbon dioxide formed. This chemical oxidation is more marked beyond the temperature range usually ascribed to the activity of micro-organisms.

LITERATURE CITED

- (1) BOEKHOUT, F. W. J., and VRIES, J. J. OTT DE
1904-15. UEBER DIE SELBSTERHITZUNG DES HEUES. Centbl. Bakt. [etc.]
(II) 12: 675-681, illus., 1904; 15: 568-573, 1905-6; 21: 398-407,
1908; 23: 106-108, 1909; 44: 290-304, illus., 1915.
- (2) BROWNE, C. A.
1929. THE SPONTANEOUS COMBUSTION OF HAY. U. S. Dept. Agr. Tech.
Bull. 141, 39 pp., illus.
- (3) BURRI, R.
1919. DIE SELBSTERHITZUNG LAGERNDER PFLANZEN MASEN MIT BESOND-
ERER BERÜCKSICHTIGUNG VON HEU UND EMD. Landw. Jahrb.
Schweiz. 33: [23]-37.
- (4) HALDANE, J. S., and MAKGILL, R. H.
1923. SPONTANEOUS COMBUSTION OF HAY. Fuel in Sci. and Pract. 11:
380-387, illus.
- (5) ——— and MAKGILL, R. H.
1934. THE SPONTANEOUS OXIDATION OF COAL AND OTHER ORGANIC SUB-
STANCES. Jour. Soc. Chem. Indus. 53: 359T-365T.
- (6) MIEHE, H.
1907. DIE SELBSTERHITZUNG DES HEUES. EINE BIOLOGISCHE STUDIE. 127
pp., illus. Jena.
- (7) ———
1911. ÜBER DIE SELBSTERHITZUNG DES HEUES. Arb. Deut. Landw. Gesell.
Heft 196, 36 pp., illus.
- (8) TRUNINGER, E.
1929. ALLGEMEINE BEOBSACHTUNGEN UND UNTERSUCHUNGEN ÜBER WESEN,
URSACHE, UND VERLAUF DER SELBSTERHITZUNG UND SELBSTENT-
ZÜNDUNG VON DURRFUTTER. Landw. Jahrb. Schweiz. 43: 278-
362, illus.

THE CONSTRUCTION OF NORMAL-YIELD AND STAND TABLES FOR EVEN-AGED TIMBER STANDS¹

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INTRODUCTION

A normal-yield table is a tabulated statement of yields to be expected from well-stocked, even-aged stands of a given timber type, at successive ages, for the range of site qualities characteristic of the type. Normal-stand tables are frequency distributions of tree stems by diameter classes within stands of given age and site quality.

All of the timber-yield tables published in the United States during the last 10 years have been based, either entirely or partially, upon the Bruce method (1)² or Reineke's modification of it (7). The accompanying stand tables have usually been based upon one of three principles of construction: (1) Correlation of percentiles of frequency with average diameter of stand (2, 8); (2) graphic fitting of either the normal curve of error or some logarithmic transformation of the normal curve to actual frequency distributions (1, 7); or (3) mathematical fitting of the Gram-Charlier series to the actual distributions after harmonizing calculated parameters with average diameter (4, 5, 9, 10).

It is the purpose of this paper to describe (1) a general method of constructing yield tables for the entire stand that is free from the inherent rigidity of the Bruce-Reineke method, and (2) a method of constructing stand tables that is more objective than the purely graphic and less laborious in calculation than the Gram-Charlier series.

YIELD TABLES

The data upon which yield and stand tables are based consist of measurements of sample plots in the desired timber type. The sample plots selected should be in even-aged stands and large enough to contain 100 to 300 trees. The objective is to enclose a comparatively complete crown canopy by excluding the larger openings which follow accident or failure of reproduction and at the same time to include the ground area equivalent to that used by the enclosed timber. After surveying plot boundaries in order to determine plot area, the following measurements are taken: (1) Diameter breast high³ of every tree by species and crown class; (2) heights of enough trees (usually 15 to 25) to permit the reliable estimate of average height of each diameter class; and (3) age counts, taken with an increment borer on several trees, to establish plot age.

From these data, the yield and the stem distribution of each plot are calculated on an acre basis. Yield is expressed in several ways, such as number of trees, basal area, average diameter, and volume in cubic and board feet. The relation of yield to age and site quality

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² Reference is made by number (italic) to Literature Cited, p. 564.

³ Four and a half feet above the ground; abbreviation, d. b. h.

is then worked out. For the western white pine type, as an example, Haig (3) constructed tables for the following expressions of yield:

- A. For the entire stand:
 - Number of trees per acre.
 - Average diameter breast high.
 - Basal area per acre.
 - Cubic-foot volume inside bark per acre.
- B. For the trees 6.6 inches d. b. h. and larger:
 - Number of trees per acre.
 - Average diameter breast high.
 - Basal area per acre.
 - Board-foot volume per acre, according to the International log rule.
- C. For the trees 7.6 inches d. b. h. and larger:
 - Board-foot volume per acre, according to the Scribner log rule.
- D. For the trees 12.6 inches d. b. h. and larger:
 - Number of trees per acre.
 - Average diameter breast high.
 - Basal area per acre.
 - Board-foot volume per acre according to the International log rule.
 - Board-foot volume per acre according to the Scribner log rule.
- E. For the dominant stand:
 - Average diameter breast high.
 - Cubic-foot volume per acre.
 - Height of average dominant (dominant and codominant) western white pine.

In Haig's investigation, as in practically all such studies, the direct relation of yield to age and site quality was worked out for the basic variables of the entire stand only, yields of the partial stand (with the exception of the height of the average dominant western white pine) being derived therefrom.

These derived curves of yield have proved workable and satisfactory. But the curves of yield of the entire stand in terms of age and site quality are forced into too rigid a mold. The nature of this rigidity will be shown and a method of analysis that entirely overcomes it described. The data consist of measurements of 99 fully stocked, even-aged sample plots of red gum (*Liquidambar styraciflua* L.) measured by R. K. Winters of the Southern Forest Experiment Station.

THE DETERMINATION OF SITE INDEX

The quantitative measure of site quality, known as site index, is the height of the average dominant (usually including the average codominant) tree at a given age, called reference age, for which 50 years is commonly taken, as in the present discussion. A necessary condition for the correct determination of site index is that it be intrinsically independent of plot age; for then the proportion of the sample plots whose site indexes are better—or poorer—than any given site index is the same, in the long run, at one age as at another, and, conversely, site index may be identified by associating it with the probability of occurrence. For example, the site qualities that are the most productive 10 percent of the area in a timber type should be represented by 10 percent of the sample plots in each age class whose height of average dominant—or dominant and codominant—tree is greatest.

The condition stated above may be fulfilled in field sampling if, while trying for approximately equal numbers of plots in each age class, conscious effort is made to secure random distribution of the sample plots with respect to site quality. It then follows that the curve of height on age (shown in fig. 1, A, for the red gum data) is

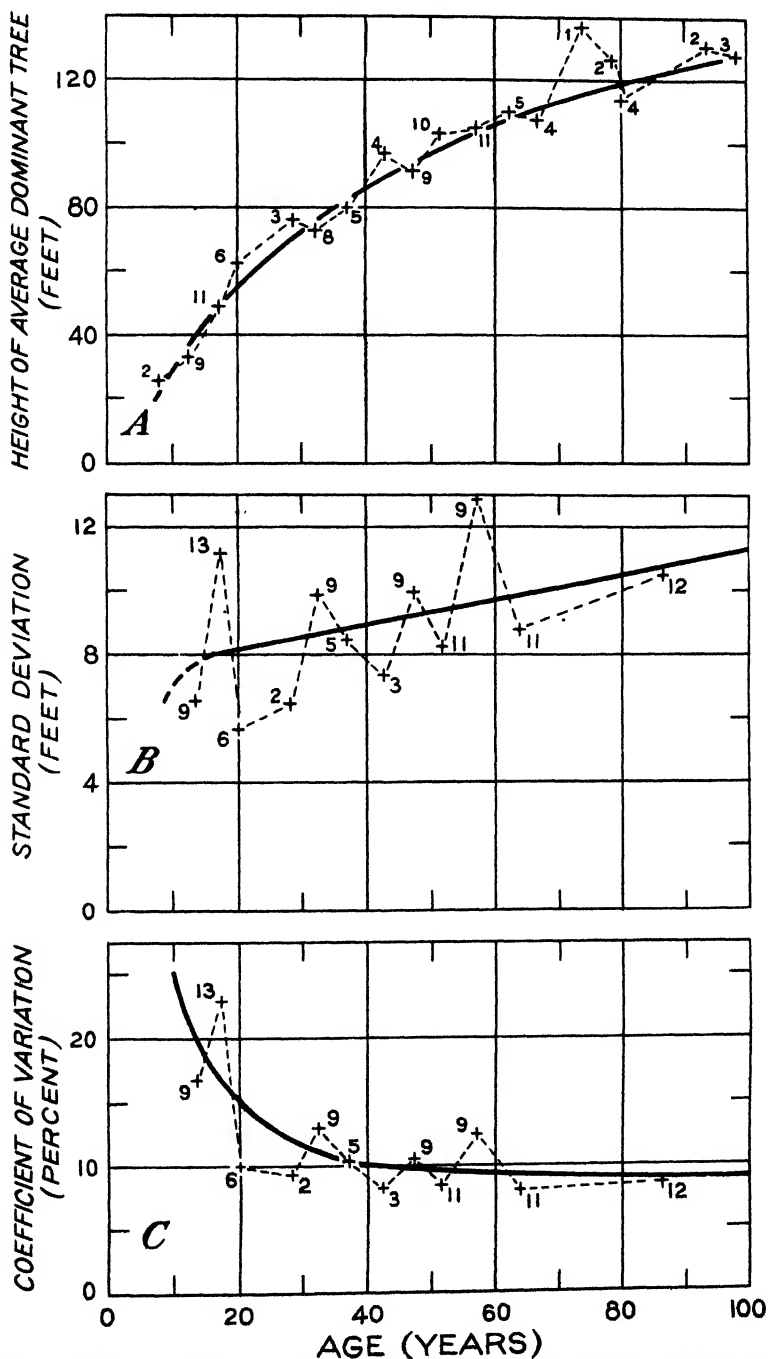


FIGURE 1.—Steps in the determination of site index: *A*, Relation of height of average dominant tree to age; since age and site index are independent of each other, this curve is also that of site index 98 feet at 50 years. *B*, Relation of the standard deviation of height of average dominant tree to age. *C*, Relation of the coefficient of variation to age.

not only the gross regression but also a partial regression, that is, it represents the relation of height to age for a constant site index as well as for the average of the site indexes, in this case 98 feet at 50 years.

The curves of height on age for other site indexes are next needed. In all of the recent yield studies in this country these have been defined as curves of the same form as that of average site index but differing therefrom by a constant ratio at all ages; for example, the curve of height of dominant stand on age for the 80-foot site index of red gum would be defined as $\left(\frac{80-98}{98}\right)$ or -0.184 ; that is, 18.4 percent less than the curve values of figure 1, *A*, at any age.

Now if site index thus defined holds true in nature, it follows that the coefficient of variation of height is independent of age. Otherwise there will not be the same proportion of the sample plots of a

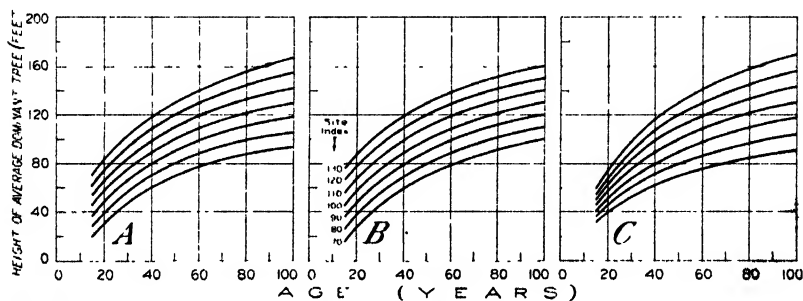


FIGURE 2.—The relation of height of average dominant tree to age and site index: *A*, As determined from the relation of the standard deviation or of the coefficient of variation to age; *B*, on the assumption that the standard deviation is independent of age; *C*, on the assumption that the coefficient of variation is independent of age.

given site class in all ages. This is an important hypothesis, and one that may be tested in each investigation. A method of making the test will be illustrated.

The distributions of residuals from the curve of the average site index of figure 1, *A*, are collected in table 1 by selected age classes. At the bottom of the table are given the standard deviations in feet, and the coefficients of variation; the latter are the standard deviations expressed as percentages of the curve value of the mean. The relation of these two constants of distribution to age is shown in figure 1, *B* and *C*. The three curves of figure 1 permit of ready cross checking, because any value of the standard-deviation curve divided by the corresponding average must equal the coefficient of variation. Since, according to the figure, the coefficient of variation is not independent of age in the early life of the stand, the definition upon which site index has generally been determined in yield studies during the past decade does not hold for the red gum data; that is, the ratio of height of dominant stand of a given site index to that of the average site index is not constant, but depends, rather, upon the age of the stand.⁴

⁴ Roy A. Chapman, of the Southern Forest Experiment Station staff, tested this hypothesis for 3 of the for which yield tables had been published; for 2 of them it did not hold.

TABLE 1.—Frequency distribution of the plot residuals from figure 1, A, by selected age classes

Deviation of height of codominant tree from curved height for aver- age site index (feet)	Plots in age class indicated											
	8-14 years	15-19 years	20-24 years	25-29 years	30-34 years	35-39 years	40-44 years	45-49 years	50-54 years	55-59 years	60-69 years	70+ years
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
+19.50 to +22.49		1										1
+16.50 to +19.49										1		
+13.50 to +16.49									1			
+10.50 to +13.49	1						1		2	1	1	
+7.50 to +10.49		1		1				1	1	1	1	
+4.50 to +7.49	1	2	6		3	1	1	2	1	1		3
+1.50 to +4.49	2	2		1	1			1	1	2	2	3
-1.50 to +1.49		1				1		2	2		3	4
-4.50 to -1.49	1	1				2	1		1	1	1	2
-7.50 to -4.49	3				2							
-10.50 to -7.49		1						1			2	
-13.50 to -10.49	1				1			2	1			
-16.50 to -13.49		3						1				
-19.50 to -16.49		1				1		1				
-22.50 to -19.49										2	1	
-25.50 to -22.49					1							1
Total	9	13	6	2	9	5	3	9	11	9	11	12
Standard deviation ¹ , feet	6.6	11.2	5.7	6.5	9.9	8.5	7.4	10.0	8.3	12.9	8.8	10.5
Curved height at average age of class, feet	39.5	49.2	56.1	70.0	76.2	82.6	89.8	95.2	99.1	104.4	109.3	123.1
Coefficient of variation percent	16.7	22.8	10.2	9.3	13.0	10.3	8.2	10.5	8.4	12.4	8.1	8.5

¹ Computed from deviations measured to the nearest foot. The data are grouped into 3-foot classes for conciseness.

Fortunately, however, the curves of height of dominant stand which result from such varying ratios may be easily deduced from figure 1. Again taking the 80-foot site index as an illustration, the curve for the height of dominant stand, as determined above, is 18.4 percent less than the curve values of figure 1, A, but this is at the reference age of 50 only. It should, however, be this same number of units of the coefficient of variation at all ages, so that the probability of occurrence of a given site index would be the same at all ages. Since the coefficient of variation (as read from figure 1, C) is 9.5 percent at 50 years, site index 80 becomes $\frac{-18.4}{9.5}$ or -1.94 units measured from the aver-

age curve. And 1.94 times the curve value of the coefficient of variation at any given age is the percentage reduction to be applied to the average curve to give the curve height for site index 80 feet at that age. The curves for other site indexes are calculated in a similar manner. Several are shown in figure 2, A.

Site index, then, may be assigned to each plot by plotting the height of its average dominant tree over its age on this graph and interpolating between the nearest curves.

In practice it is perhaps simpler to base the site-index curves upon the standard deviation rather than upon the coefficient of variation. As site index is independent of age, though the standard deviation of dominant height may or may not be independent of age, site index may be defined as a deviation in standard units from the curve of dominant height on age. The absolute value of a standard unit at

any age for red gum is given in figure 1, *B*. For the reference age of 50 years it is 9.3 feet. Hence the 80-foot site index curve is $\left(\frac{80-98}{9.3}\right) = -1.94$ standard units measured from the curve of figure 1, *A*. At 100 years of age, for instance, the height of dominant stand for site index 80 feet is $130 + (-1.94 \times 11.3) = 108$ feet. This process suggests a general expression of height of dominant stand in terms of age and site index. We have (1) the expression

$$H = f_1(A)$$

where *H* is height of dominant stand for average site index, expressed as a function f_1 of age *A* (fig. 1, *A*); (2) the standard deviation σ_H of the height of dominant stand as a function of age *A*, that is,

$$\sigma_H = f_2(A)$$

where f_2 is such a function as defined in figure 1, *B*. From these two expressions and from the definition of site index as given above,

$$H = f_1(A) + f_2(A) \left\{ \frac{S - f_1(A_R)}{f_2(A_R)} \right\}$$

in which *H* is height of dominant stand at age *A* for site index *S*, and A_R is the reference age which determines the numerical expression of site index. This is a general expression of the joint effect of two independent variables upon a dependent variable when the correlation between the independents is zero.

If the standard deviation had been found to be independent of age, a series of site-index curves, such as those of figure 2, *B*, would have resulted, in which each is a constant difference from any other. On the other hand, had the coefficient of variation been independent of age, the result would have been a series of site-index curves, each a constant ratio of any other (fig. 2, *C*). Since, however, both the standard deviation and the coefficient of variation are dependent upon age, the best estimate, as shown in figure 2, *A*, is intermediate between the others.

THE CONSTRUCTION OF YIELD TABLES FOR THE ENTIRE STAND

The same general method may be used for the expression of entire stand yield in terms of age and site index. Since age and site index are independent of each other, the gross regression of yield on age is also the partial regression for the average of all site indexes present. For a particular site index, however, the regression of yield on age is (in the general case) at a level which differs from the gross regression by a certain number of units of the standard deviation of yield around the gross regression. How the number of such standard units corresponding to each site index is defined may be illustrated through the analysis of yield of red gum in cubic feet and in number of trees.

In figure 3 is shown the relation to age of the gross regression of volume, the standard deviation of volume, and the coefficient of variation of volume. The last-named is primarily for cross checking with the other two. The plotted points were computed in the same way as and the curves are analogous to those of figure 1.

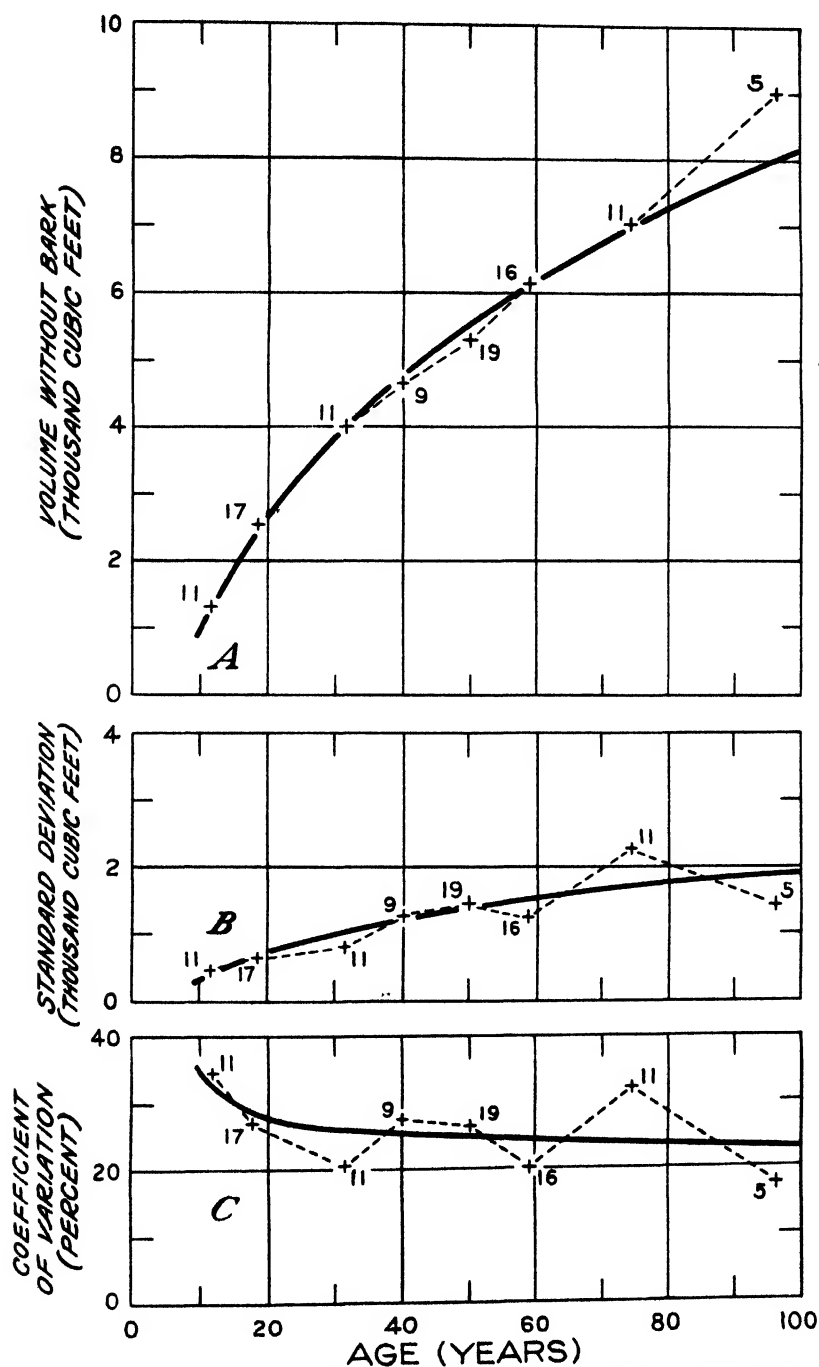


FIGURE 3.—Steps in the correlation of volume with age and site index: A, Relation of volume per acre to age for the average of the site indexes; B, relation of the standard deviation of volume to age; C, relation of the coefficient of variation of volume to age.

The sample-plot data are next sorted according to site-index classes and entered on a form such as table 2, which shows a partial listing of the data for red gum, site index class 70-74 feet. In column 5 are given the estimated volumes of the plots according to plot age, taken from figure 3, *A*. The residuals are given in column 6. In column 7 are the estimated standard deviations according to age as read from figure 3, *B*, and in column 8 the ratio of the residual to the standard deviation. For each site-index class the average site index and the average of the corresponding standard units are then computed. These standard units are then plotted on site index, and a freehand curve fitted to the points (fig. 4, *A*), the curve defining the deviation of the volume curve of any site index from the

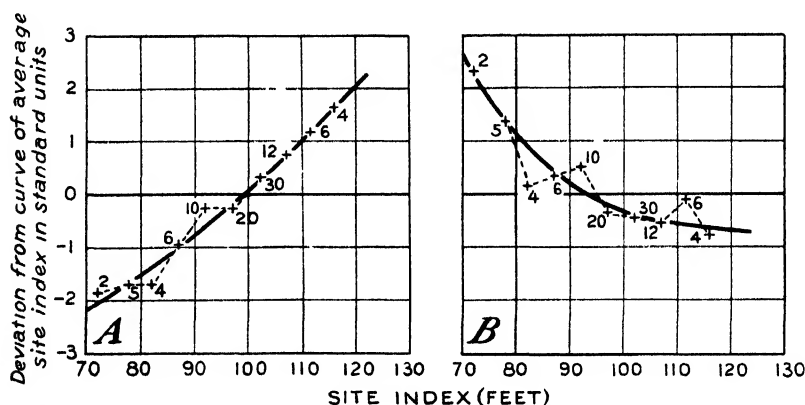


FIGURE 4.—The partial regression of yield (in units of the standard deviation at any age) on site index for *A*, volume in cubic feet, and *B*, number of trees.

volume curve for the average of the site indexes, in standard units of volume at a particular age. It is converted to units of volume by multiplying by the standard deviation for the age.

TABLE 2.—Form of compilation and calculations preliminary to the determination of the net regression curves of volume in cubic feet on age

Site-index class (feet)	Site index	Age	Volume per acre			Standard deviation at indicated age	Actual minus curved + standard deviation
			Actual	Curved (for the average site index)	Actual minus curved		
	<i>Feet</i>	<i>Years</i>	<i>M cu. ft.</i>	<i>M cu. ft.</i>	<i>M cu. ft.</i>	<i>M cu. ft.</i>	<i>Standard units</i>
70-74.....	70	31	1.18	3.95	-2.77	1.02	-2.71
	74	56	3.37	5.92	-2.55	1.46	-1.75
Sum.....	144						-4.46
Average.....	72						-2.23

To summarize, three relationships have been established: (1) The expression of volume for the average of the site indexes, in terms of age (fig. 3, *A*) represented by

$$V=f_1(A)$$

in which V is volume, f_1 is its expression in terms of age (A) for the average of site indexes; (2) the standard deviation of volume in terms of age (fig. 3, B), or

$$\sigma_v = f_2(A)$$

in which σ_v is the standard deviation of volume, and f_2 is its expression in terms of age (A); and (3) from figure 4, A —

$$\frac{v}{\sigma_v} = f_3(S)$$

in which v is the deviation of the volume for a given site index from $f_1(A)$, σ_v has the same meaning as above, and f_3 is the expression of the ratio in terms of site index (S). The volume for any age and site index may therefore be expressed as follows:

$$V = f_1(A) + \{f_2(A) \cdot f_3(S)\}$$

In figures 5 and 4, B , are presented the set of curves needed to express number of trees per acre in terms of age and site index. It is preferable, in the case of this variable, to plot both the average number of trees and the standard deviation on logarithmic scale, as the same relative accuracy is thus assured at all ages.

As stated above, the methods now in use for arriving at the yields of the partial stands have proven workable and satisfactory. These methods are based upon the relation of one of the following values of the partial stand to the average diameter breast high of the entire stand: (1) The ratio of the partial stand yield to the entire stand yield, such as the ratio of the basal area of the trees 6.6 inches d. b. h. and larger to the basal area of the entire stand, or the ratio of board-foot volume in the trees 12.6 inches d. b. h. and larger to the cubic-foot volume of the entire stand; (2) the difference between the partial stand yield and the corresponding yield of the entire stand, such as the difference between the average diameter breast high of the trees more than 12.5 inches and the average diameter breast high of the entire stand.

The yields for the partial stand are calculated by applying the proper ratio or difference to the particular yield of the entire stand which had served at the base of the ratio or difference, by reference to the average diameter breast high of the entire stand.

While this method is subject to a theoretical imperfection in that the ratios or differences as related to the average diameter breast high of the entire stand have not been proven to be completely independent of age and site quality, it has two important practical advantages: (1) Proper weights may be readily assigned to ratios which are correlated with only a single variable; (2) the correlation of ratios, or differences, with average diameter breast high serves to prevent absurdities which may follow the fitting of yield curves independent of one another to age and site quality.⁵

⁵ It is obvious, for instance, that the average diameter breast high of the trees 6.6 inches and larger cannot be less than the average diameter breast high of the entire stand; that the ratio of the basal area of a partial stand to the basal area of the entire stand cannot exceed 1.

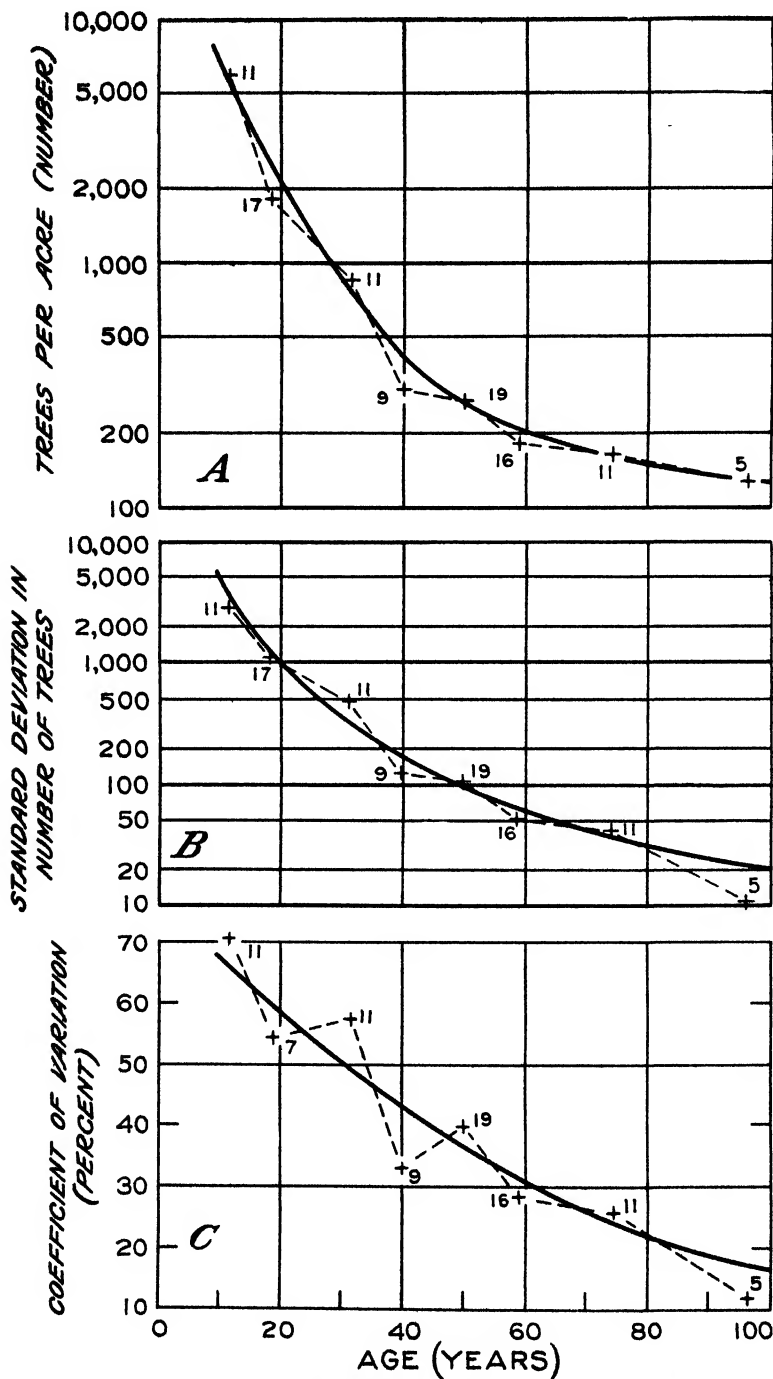


FIGURE 5.—Steps in the correlation of number of trees with age and site index: *A*, Relation of number of trees per acre to age for the average of the site indexes; *B*, relation of the standard deviation of the number of trees to age; *C*, relation of the coefficient of variation to age.

STAND TABLES DEFINED BY THE PEARL-REED POPULATION GROWTH CURVE

The value of an objective method of stand-table construction is apparent. It should be flexible enough to maintain the inherent variation of timber stands, but it should not necessitate laborious computations in application. The search for such a method led to the Pearl-Reed population growth curve (6). This curve is of the form

$$y=c+\frac{k}{1+me^{f(x)}} \quad (1)$$

where y is the population in a restricted geographical area, c is the lower asymptote or the population at the beginning of a cultural epoch, $(k+c)$ is the upper limiting asymptote of population, m is an

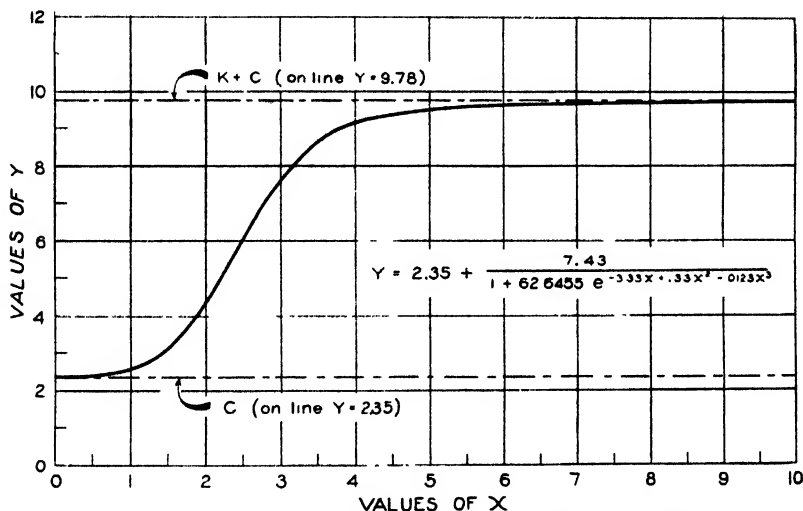


FIGURE 6—An illustration of the Pearl-Reed population growth curve.

arbitrary constant, e is the base of the Naperian logarithms, x is the date, and $f(x)$ is of the form $b_1x+b_2x^2+\dots+b_nx^n$. Figure 6 is an example of this type of curve.

A special case occurs when $c=0$ and $k=100$. If, under these conditions, y is defined as the number of trees in an even-aged timber stand whose diameters are less than a given limit x , expressed in percentage of the total number of trees in the stand; then the curve of equation (1) closely resembles the cumulative frequency curve of a typical forest-stand diameter distribution (fig. 7).

In an analysis of a curve of this type it is convenient to use the calculus method. A reader not interested in the theory may pass over the subsequent discussion as far as equation (2) without loss of continuity.

The curves of figure 7 approach the ordinates $y=0$ and $y=100$ very slowly; hence these ordinates may be considered asymptotes to the curves. This suggests the condition—

$$\frac{dy}{dx} = (100-y)y f(x)$$

for which $\frac{dy}{dx}$ vanishes at $y=0$ and at $y=100$. To facilitate the integration, the right-hand member may be divided by -100 . This operation does not change the values of y when $\frac{dy}{dx}=0$. Accordingly,

$$\frac{dy}{dx} = \frac{(100-y)y}{-100} f(x).$$

Separating variables, we have—

$$\frac{-100dy}{(100-y)y} = f(x)dx.$$

Adding and subtracting $\frac{ydy}{(100-y)y}$, this is reduced to—

$$\frac{-dy}{100-y} - \frac{dy}{y} = f(x)dx.$$

Integrating—

$$\int \frac{-dy}{100-y} - \int \frac{dy}{y} = \int f(x)dx + a$$

or

$$\log\left\{\frac{100-y}{y}\right\} = f'(x) + a.$$

If we define

$$f'(x) = b_1x + b_2x^2 + b_3x^3 + \dots + b_nx^n$$

then

$$\log\left\{\frac{100-y}{y}\right\} = a + b_1x + b_2x^2 + \dots + b_nx^n. \quad (2)$$

The curve is most easily fitted to data in the form of equation (2). Its exponential form is—

$$\frac{100-y}{y} = e^{a+b_1x+b_2x^2+\dots+b_nx^n}$$

or

$$y = \frac{100}{1 + e^{a+b_1x+b_2x^2+\dots+b_nx^n}} \quad (3)$$

and this is identical with the Pearl-Reed curve when $c=0$, $k=100$, and $m=e^a$.

In order to determine the number of terms in x necessary to fit equation (2) satisfactorily to even-aged stands as encountered,

coordinates were read from a Pearson type III curve, the β_1 and β_2 coefficients of which were 1.73 and 4.96 respectively; and the following equations fitted successively:

$$\log\left\{\frac{100-y}{y}\right\}=a+bx$$

$$\log\left\{\frac{100-y}{y}\right\}=a+b_1x+b_2x^2$$

$$\log\left\{\frac{100-y}{y}\right\}=a+b_1x+b_2x^2+b_3x^3$$

The first two proved inadequate but the third followed the data closely. This is deemed sufficient reason to apply the last form to the red gum distributions.

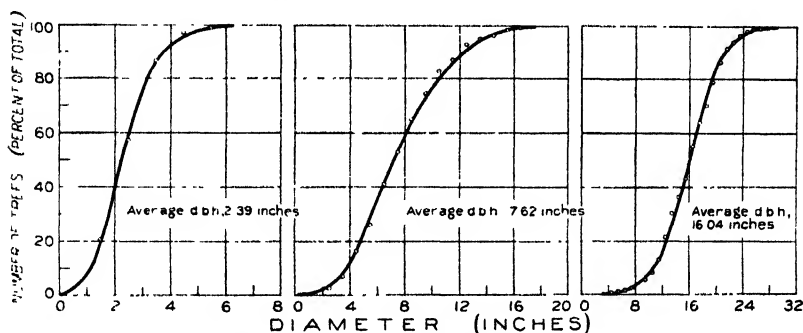


FIGURE 7.—Comparison of actual with calculated cumulative frequencies in stands of small (A), medium (B), and large (C) timber.

APPLICATION TO INDIVIDUAL STANDS

The plots were therefore sorted according to average diameter (by basal area) into groups with $\frac{1}{2}$ -inch class intervals. A combined stand tally for each group was then computed, showing the frequency of trees in each 1-inch diameter class from which the arithmetic average of the diameters was calculated. The frequencies of successive diameters, starting with the lowest, were then accumulated, and the cumulative values expressed as percentages of the total frequency. Table 3 shows the form of this summary, a line for each group.

TABLE 3.—Form of summarizing field data on forest stands

Average stand diameter class by basal area (inches)	Arithmetic average diameter breast high	Plots	Cumulative frequency percentages (y) for diameter class limits (upper), inches (x)							
			1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5
2.5-2.9	Inches 2.39	Number 5	Percent 20.6	Percent 57.4	Percent 86.8	Percent 97.0	Percent 99.0	Percent 99.6	Percent 100	Percent 100
3.0-3.4	Inches 2.93	Number 3	Percent 10.8	Percent 46.0	Percent 72.0	Percent 87.1	Percent 95.0	Percent 98.1	Percent 99.5	Percent 100

Defining y as the cumulative frequency to the upper limit of any diameter class, and x as the corresponding upper limit itself, a curve of the form of equation (2) in which—

$$\log\left\{\frac{100-y}{y}\right\}=a+b_1x+b_2x^2+b_3x^3$$

was fitted to the data of each group by the method of least squares, the constants a , b_1 , b_2 , and b_3 being derived from the solution. In this work the y values were given unit weight and the x values were assumed to be without error.

The actual data of each group and the derived curve were then plotted on the same graph. Three of these comparisons are shown in figure 7. After completing this work for each of the 27 groups, the constants a , b_1 , b_2 , and b_3 were plotted over the arithmetic average diameter breast high of the group as in figure 8.

ADJUSTMENT OF THE CONSTANTS

The distribution constants plotted in figure 8 should be adjusted in such a way that they progress smoothly with increase in average diameter breast high and also that they be consistent with one another. To effect the required harmonization a method of successive approximation was adopted which permits of the adjustment of two of the constants at a time, through the following relationships:

$$\left. \begin{aligned} na+b_1\Sigma x+b_2\Sigma x^2+b_3\Sigma x^3 &= \Sigma \log\left(\frac{100-y}{y}\right) \\ a+b_1M_d+b_2M_d^2+b_3M_d^3 &= 0 \end{aligned} \right\} \quad (4)$$

in which n is the number of diameter classes in an actual distribution and M_d is the median diameter breast high of the computed curve. The first equation is simply one of summation over all diameters of an actual distribution. The second equation, based upon the definition of the median, is simply that $y=50$ when x =median diameter breast high; that is—

$$\log\left(\frac{100-y}{y}\right)=\log\left(\frac{100-50}{50}\right)=\log 1=0$$

at median diameter breast high. Accordingly, the median diameter was calculated from each curve. The relation of the median diameter to the arithmetic average diameter itself is shown in figure 9. By means of this relation the median diameter breast high for the second of equations (4) is determined.

Since the data of figure 8 define trends of the constants b_2 and b_3 that are obviously more definite than those of a and b_1 , freehand curves representing second estimates of the former two constants were drawn as shown. The curve values of each group were then substituted for b_2 and b_3 in equations (4), and the corresponding medians taken from figure 9, as stated above. The simultaneous solution of equations (4) for each group thus affords second estimates of the constants a and b_1 which have well-defined trends and considerably less variation than the first estimates; these were used in drawing the trend curves shown in the upper half of figure 8.

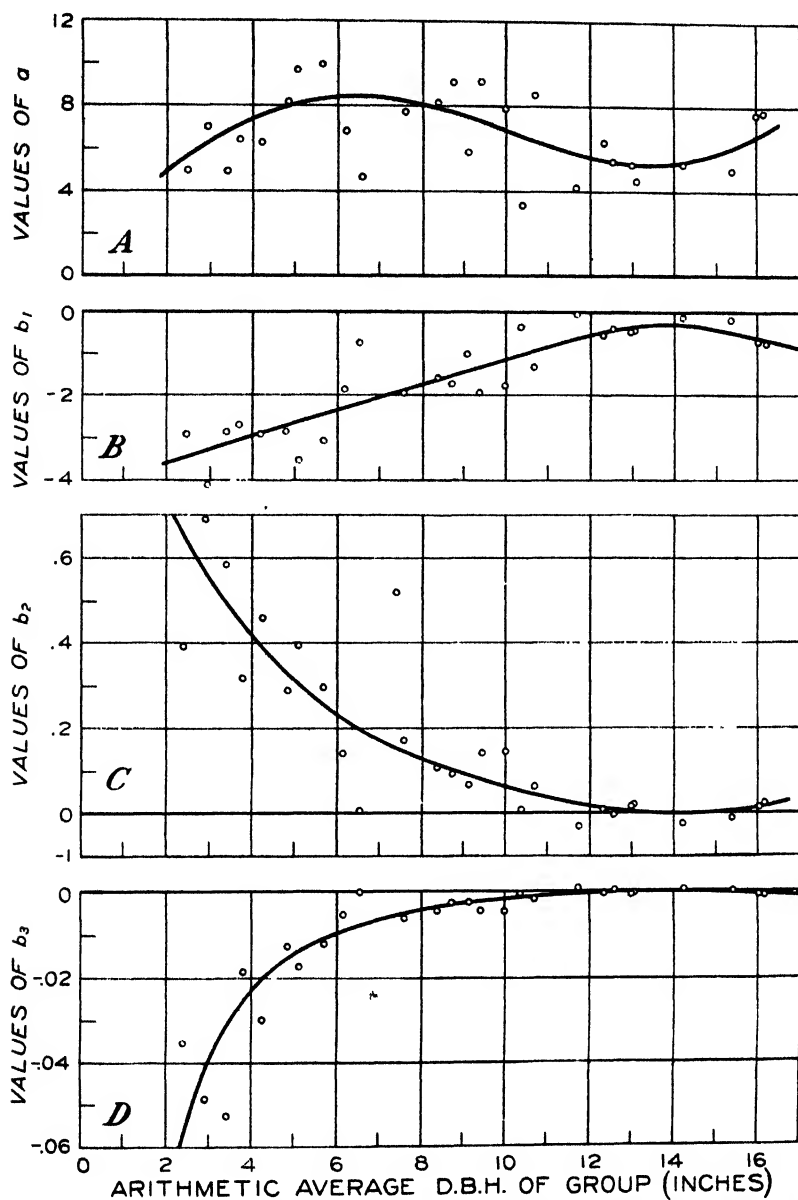


FIGURE 8.—Steps in the adjustment of cumulative frequency constants. The plotted points, called first estimates, were calculated for each group. Freehand curves through b_2 and b_1 (C, D), called second estimates of these constants, were used to calculate second estimates of a and b_1 (A, B); through the latter freehand curves were drawn, and these are compared with the first estimates of a and b_1 (A, B).

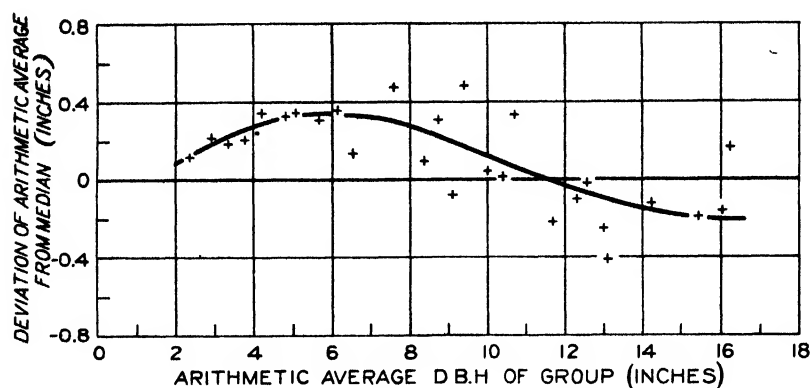


FIGURE 9.—The deviation of the arithmetic average diameter breast high from median diameter breast high as related to the arithmetic average diameter of each group.

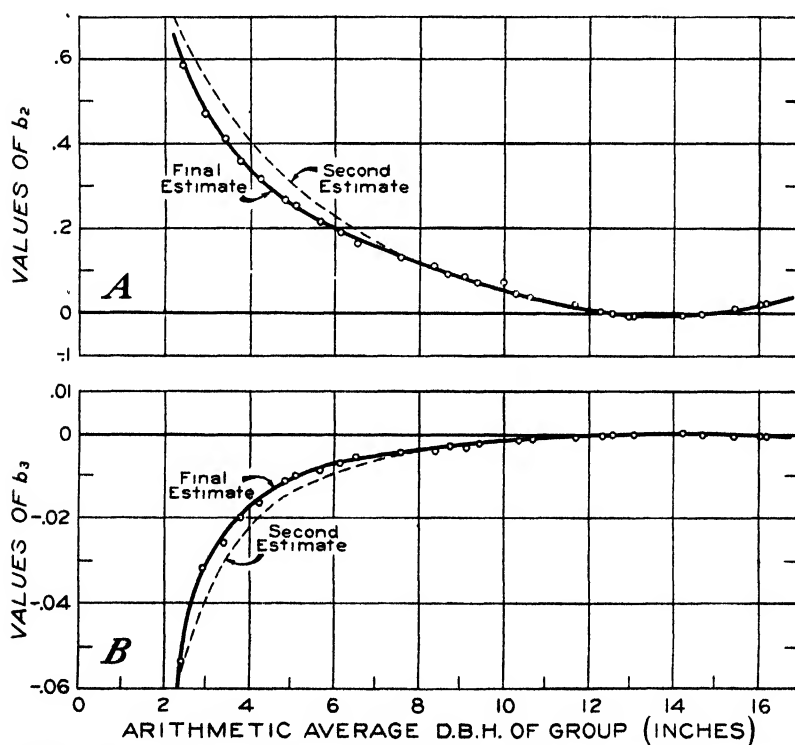


FIGURE 10.—Final adjustment of cumulative frequency constants b_2 and b_3 at A and B respectively.

As a check, the new curve estimates of a and b_1 were substituted in equations (4), and new values of b_2 and b_3 calculated. In figure 10 these are compared graphically with the previous estimates. Slight changes were made as indicated.

In order to retain three figures for the numerical values of b_2 and b_3 , the curves of figure 10 were redrawn on semilogarithmic paper, with breaks, of course, as b_2 becomes negative and b_3 approaches zero. These final adjusted constants permit the calculation of such cumulative stand tables as may be desired, by substituting them in equation (2) or (3). One for each inch of average diameter breast high by basal area is presented in figure 11. Since the constants are expressed in terms of the arithmetic average diameter of the 27 original groups (figs. 8 and 10), a subsidiary curve of the latter on average diameter by basal area was first constructed, and from this, the arithmetic average diameter corresponding to each whole inch of average diameter by basal area was read, and the associated constants taken from figures 9 and 10 as redrawn on logarithmic scale.

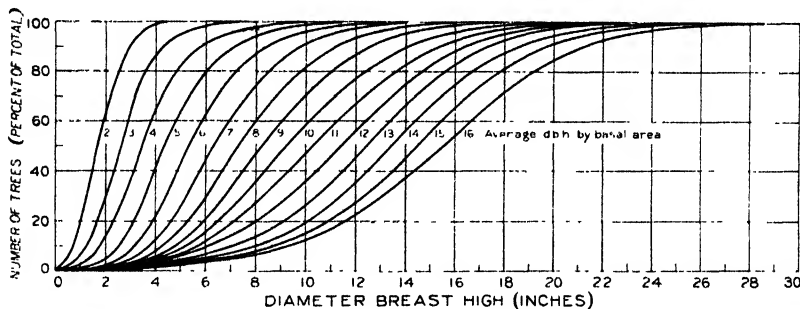


FIGURE 11.—Cumulative frequency percentages in number of trees by diameter-breast-high classes for red gum.

SUMMARY

A method of constructing normal-yield tables is described which does not presuppose, as the Bruce-Reineke method does, that the coefficient of variation of yield of the entire stand is the same at all ages. It is shown, using normal stands of red gum as examples, that the coefficient of variation for height of dominant stand, for volume, and for number of trees is dependent upon age of stand.

The basis of the method is that the relation of the standard deviation, or the coefficient of variation of yield, to stand age determines the form of the growth curve of any site index from the growth curve of the average site index.

Stand tables are constructed by application of the Pearl-Reed population growth curve to cumulative frequency distributions of red gum by diameter-breast-high classes.

A method of harmonizing the curves through adjustment of the descriptive constants two at a time, by successive approximation, is described. The work is less laborious than the use of the Gram-Charlier series and is more objective than the strictly graphic methods of constructing stand tables.

LITERATURE CITED

- (1) BRUCE, D.
1926. A METHOD OF PREPARING TIMBER-YIELD TABLES. *Jour. Agr. Research* 32: 543-557, illus.
- (2) ——— and REINEKE, L. H.
1929. THE USE OF ALINEMENT CHARTS IN CONSTRUCTING FOREST STAND TABLES. *Jour. Agr. Research* 38: 289-308, illus.
- (3) HAIG, I. T.
1932. SECOND-GROWTH YIELD, STAND, AND VOLUME TABLES FOR WESTERN WHITE PINE TYPE. U. S. Dept. Agr. Tech. Bull. 323, 68 pp., illus.
- (4) MEYER, W. H.
1928. RATES OF GROWTH OF IMMATURE DOUGLAS FIR AS SHOWN BY PERIODIC MEASUREMENTS OF PERMANENT SAMPLE PLOTS. *Jour. Agr. Research* 36: 193-215, illus.
- (5) ———
1930. DIAMETER DISTRIBUTION SERIES IN EVENAGED FOREST STANDS. *Yale Univ. School Forestry Bull.* 28, 105 pp., illus.
- (6) PEARL, R., and REED, L. J.
1923. ON THE MATHEMATICAL THEORY OF POPULATION GROWTH. *Metron* 3 (1): [6]-19, illus.
- (7) REINEKE, L. H.
1927. A MODIFICATION OF BRUCE'S METHOD OF PREPARING TIMBER-YIELD TABLES. *Jour. Agr. Research* 35: 843-856, illus.
- (8) SCHUMACHER, F. X.
1926. YIELD, STAND, AND VOLUME TABLES FOR WHITE FIR IN THE CALIFORNIA PINE REGION. *Calif. Agr. Expt. Sta. Bull.* 407, 26 pp., illus.
- (9) ———
1928. YIELD, STAND, AND VOLUME TABLES FOR RED FIR IN CALIFORNIA. *Calif. Agr. Expt. Sta. Bull.* 456, 29 pp., illus.
- (10) ———
1930. YIELD, STAND, AND VOLUME TABLES FOR DOUGLAS FIR IN CALIFORNIA. *Calif. Agr. Expt. Sta. Bull.* 491, 41 pp., illus.

PHYSIOLOGICAL FACTORS INFLUENCING THE RATE OF EGG FORMATION IN THE DOMESTIC HEN¹

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INTRODUCTION

It is a well-established fact that a bird's annual egg record is conditioned by a number of more or less independent factors. Some of these factors are age at sexual maturity, pauses in production due to broodiness or other inherent tendencies, persistency, and intensity (rate of production).

One of the most important members of the group of factors is rate of production. Hays² has presented some evidence that this trait is inherited. The usual measures of intensity or rate of production have been percentage of egg production for a given period or the mean clutch size. Hays and Sanborn³ have shown that either may be used as a measure of intensity. Considerable attention has been given to the hereditary but very little to the physiological aspects of intensity. It is the purpose of this paper to consider the physiological basis of intensity of egg production. A more adequate knowledge of these features should aid greatly in the genetic approach to the somewhat complicated problem of heredity of rate of egg production.

EXPERIMENTAL FINDINGS

RELATION OF PRODUCTION RATE TO INTERVAL AND CLUTCH

This study is concerned primarily with those features which influence the rate of egg formation. It is a well-recognized fact that individual hens have a characteristic time interval between eggs which is maintained with fair uniformity. The daily egg record is characterized by a succession of groups of consecutive eggs of more or less uniform number separated by a day's pause. The series of eggs on successive days has usually been referred to as a clutch. The rate of production is dependent largely upon the size of the clutch or in other words, the frequency with which the days of nonproduction occur.

Accurate timing of egg laying of individual birds reveals the fact that the length of the interval between successive eggs is an important factor in determining clutch size. The first egg of a clutch is usually laid in the morning and since the interval is ordinarily more than 24 hours the successive eggs are produced somewhat later each day. Thus a bird with a 26-hour interval may lay at 8 a. m., 10 a. m., 12 noon, 2 p. m., 4 p. m., and then miss a day's production. Then the clutch will be repeated after a day's rest. Birds with longer intervals will have smaller clutches. This relationship between interval length and clutch size has been well established by Atwood.⁴

¹ Received for publication June 1, 1935, issued December 1935. Contribution no. 88 from the Department of Poultry Husbandry, Kansas Agricultural Experiment Station.

² HAYS, F. A. INBREEDING OF RHODE ISLAND RED FOWL WITH SPECIAL REFERENCE TO WINTER EGG PRODUCTION (PRELIMINARY REPORT). Amer. Nat. 58: 49-59. 1924.

³ ——— and SANBORN, R. INTENSITY OR RATE OF LAYING IN RELATION TO FECUNDITY. Mass. Agr. Expt. Sta. Tech. Bull. 11, pp. 180-194. 1927.

⁴ ATWOOD, H. A STUDY OF THE TIME FACTOR IN EGG PRODUCTION. W. Va. Agr. Exp. Sta. Bul. 223, 11 pp. 1929.

It is known that there is some relationship between egg production and daylight and darkness since hens do not lay at night and the onset of darkness in some cases appears to be the factor responsible for the termination of the clutch. Previous investigators have believed that the egg which, due to the characteristic interval length, would have been laid in the latter part of the afternoon or at night had been formed normally but, because of the onset of darkness, was not laid at the end of the usual interval. The usual interpretation was that the egg was fully formed at the end of the normal interval but withheld by the hen until the next morning, thus becoming the first egg of the new clutch. Thus if a bird laid at 4 o'clock yesterday and had an interval length of 28 hours she would be due to lay at 8 o'clock tonight. It was thought that today's egg would be fully formed at 8 o'clock but because of darkness would be held until the next morning. If this were the correct interpretation, the egg following a day's pause (the first egg of the clutch) would be in the oviduct much longer than other eggs. Such eggs have been referred to in the literature as held eggs.

It is now known that there is no withholding of the first egg of the clutch and that the formation time is the same regardless of clutch position. The missing of a day between clutches is due to a delay in ovulation instead of the withholding in the uterus of the fully formed egg. This fact was established by bihourly examinations of a number of hens during several days. The position and stage of formation of each egg was determined by handling the hens individually. Figure 1 brings out the fact that the eggs following a pause require no more time for formation than do those laid consecutively. The record shown in figure 1 was obtained under normal lighting conditions.

TIMING EGG FORMATION

In addition to studies from autopsies, time records were secured⁵ on five anesthetized hens wherein it was possible to follow the formation of the egg from time of ovulation to its entry into the uterus. From these studies no differences in rate of passage through the oviduct could be detected in hens differing in interval length. Although the number of birds was small this was interpreted to indicate that differences in interval length probably may be accounted for largely by the time spent by the egg in the uterine region of the oviduct.

To secure additional data on the time spent by the egg in different parts of the oviduct of hens varying in interval length, observations were made on two groups of hens. The hens were White Leghorns, Rhode Island Reds, and a few first-generation hybrids. They were kept in individual hen batteries to expedite frequent examination. Two series of 40 hens each were observed for periods of 2 weeks each. The hens were examined at 2-hour intervals from 7 a. m. until 9 p. m. and egg-laying records were taken hourly throughout the day. At each 2-hour interval the position of the egg was determined by probing the cloacal opening. Data were taken on both the position

⁵ WARREN, D. C., and SCOTT, H. M. OVULATION IN THE DOMESTIC HEN. *Science* (n. s.) 80: 461-462 1934.

— and SCOTT, H. M. THE TIME FACTOR IN EGG FORMATION. *Poultry Sci.* 14: 195-207. 1935.

JAN.7	M		MP		MP		MP		MP		SS	SS	H
8	H	X	O		O		M		M		MP	MP	H
9	H		H		H		H	X	O		O	O	O
10	O		O		M		MP		MP		SS	H	H
11	H	X	O		O		O		M		MP	MP	H
12	H		H		O		⊗		O		O	O	O
13	O		M		M		MP		MP		SS	H	H
14	H		⊗		O		O		M		MP	MP	H
15	H		H		⊗		O		O		M	M	MP
16	H		H		H		H		H		⊗	O	O
17	O		O		M		MP		MP		MP	H	H
18	H		H	X	O		O		M		MP	MP	SS
19	H		H		H		H	X	O		O	O	O
20	O		O		M		MP		MP		MP	MP	H
	7	8	9	10	11	12	1	2	3	4	5	7	9
	AM						PM						
	TIME OF EXAMINATION												

FIGURE 1.—Record sheet of bird no. 34 for a 2-week experimental period in which each egg was traced during formation. The hen was examined at the hours indicated and the stage of egg formation recorded. The letters and symbols record the following stages: M, Membranous egg; MP, membranous egg well plumped, SS, soft shell; H, hard shell; X, egg laid since last examination; ⊗, egg laid since last examination and no evidence of the succeeding egg; O, no evidence of egg in oviduct. Thus on January 13, no evidence of an egg was found at 7 o'clock but a membranous one was found at 9. The egg became plump at 1 o'clock, was soft shelled at 5, and hard shelled at 7. It was hard shelled the next morning at 7 and was laid between the 8- and 9-o'clock examination. The succeeding egg was not found in the oviduct at the 9-o'clock examination on January 14.

of the egg and the stage of formation. The presence of the egg could be detected first at about the time it entered the isthmus. At this early stage the egg could be located only by probing into the large intestine and exploring the region near the dorsal abdominal wall. Experience gained in following a few eggs made it possible to make relatively accurate determinations of position. Records were taken as to the plumpness and degree of shell formation of the egg.

The positions and stages of development utilized in following the egg in the process of formation were: Time of laying of previous egg, first detection of the egg (approximate time of entering the isthmus), plumping of egg after about 2 hours in the uterus, and the beginning of shell deposition. By these observations a comparison could be made of the rate of passage through the oviducts of hens differing widely in length of interval.

A sample record sheet for one hen subjected to normal lighting during the 2-week examination period is given in figure 1. From record sheets such as this, summaries were made (table 1) of the data secured for 2 weeks on the 80 birds under observation. In a preliminary experiment the hens were handled hourly throughout the day, but it was found that such frequent disturbance caused most of the birds to stop laying. The interval between handlings was increased to 2 hours and most of the hens remained in production under these conditions. It was believed that the birds ceased to lay because of the frequent disturbance rather than because of any injury resulting from the exploration of the abdominal cavity.

In the presentation of the data in table 1 an attempt has been made to account for differences in interval length in terms of time spent in different parts of the oviduct.

TABLE 1.—Mean time spent in different parts of the oviduct by eggs requiring various periods for formation

Item	Time required for interval lengths ¹ of—					
	25	26	27	28	29	30
Time from laying of previous egg to entrance of isthmus by next egg.....	Hours 4.3	Hours 4.6	Hours 4.2	Hours 4.7	Hours 5.2	Hours 5.3
Time spent in uterus.....	18.0	18.4	19.9	19.8	20.8	21.6
Time from first indication of shell until egg is laid.....	13.8	14.7	15.6	16.4	17.0	17.9
Eggs laid.....	Number 9	Number 25	Number 20	Number 42	Number 44	Number 21

¹ Much of the 5-hour difference in interval length observed here is accounted for in a lengthening of the absolute time spent in the uterus.

FORMATION STAGES AND PERIODS STUDIED

The timed observations made it possible to establish four rather definite stages in egg formation. By using these stages as boundaries it was possible to divide the process of formation into two periods. The stages which the records revealed were: (1) Time of laying of previous egg; (2) first detection of the forming egg about the time it entered the isthmus; (3) the plumping of the egg which occurred about 2 hours after entering the uterus; and (4) the laying of the egg being traced.

The first period included the time between laying and ovulation and also included roughly the time spent in the magnum (albumen-

secreting section). If the egg is the first one of a new clutch this period also includes the extended interval responsible for the interruption in production and the establishment of clutches. Therefore, with the first egg of the clutch, the period intervening between the previous laying and the detection of the succeeding egg has been used as a measure of the delay in ovulation, since the time spent by the egg in the magnum before discovery constitutes only a small part of the whole. When no delay in ovulation occurred, the time between expulsion of the previous egg and ovulation of the next one was so small that the period was considered primarily as a measure of the time spent in the magnum.

The second period was the one between the egg's attainment of a plump stage and its expulsion. This constituted roughly the time spent in the uterus or the period in which shell deposition occurred. It is realized that some of the limits of these periods have been somewhat inaccurately established by the methods of observation utilized but they do offer an avenue of approach to the problem of physiological factors influencing rate of production which would otherwise not be available. Since a large enough number of eggs have been traced to make it possible to express comparisons as means the errors of observation probably have been largely compensated.

INTERVAL AND TIME SPENT IN DIFFERENT REGIONS OF OVIDUCT

In table 1 the data have been segregated on the basis of the interval required for egg formation. The term "interval" is used in this paper to apply to the time ensuing between the laying of successive eggs of the clutch. Thus the interval is a fairly accurate measure of the time required for egg formation. Observations on first eggs of the clutch have been excluded from these data because no accurate knowledge of the time spent in the oviduct could be secured. Due to the delay in ovulation occurring in the case of these eggs the time of the previous laying is of no value in calculating the time required for egg formation. For those eggs following the first of the clutch there intervenes only a few minutes between laying of the one egg and the ovulation of the next. Therefore, in eggs other than the first one of the clutch the time spent in the oviduct and the interval length are practically identical. The data have been so interpreted in calculating the time spent in the various regions of the oviduct in eggs varying in interval length.

The range of period required for egg formation (interval length) in the 161 eggs traced, was from 25 to 30 hours. Many of the eggs produced were not included in this total. All eggs following a pause, and any others where data were incomplete were excluded. Some birds stopped laying during the experiment while others produced many one-egg clutches which were of no value. By calculating the mean time spent in the various parts of the oviduct by the eggs showing variation in interval length, one should be able to determine in what part of the oviduct the extra time is spent by the eggs with long intervals. This should indicate how high- and low-intensity birds differ in egg formation. Intensity of egg production is conditioned by two factors, the interval length and the length of the pause between clutches. In the following discussion those factors responsible for variations in interval length are considered first.

REGIONAL DISTRIBUTION OF VARIATIONS IN INTERVAL LENGTH

It is seen that the extreme range of variations (table 1) in time from laying of previous egg to the entrance of the next egg into the isthmus will account for only a small part of the absolute difference in interval length encountered. The variation in time recorded between laying and the reaching of the isthmus by the next egg was only 1 hour (4.3 to 5.3 hours) while the variation in interval length was 5 hours (25 to 30 hours). There remains the question whether the variation in

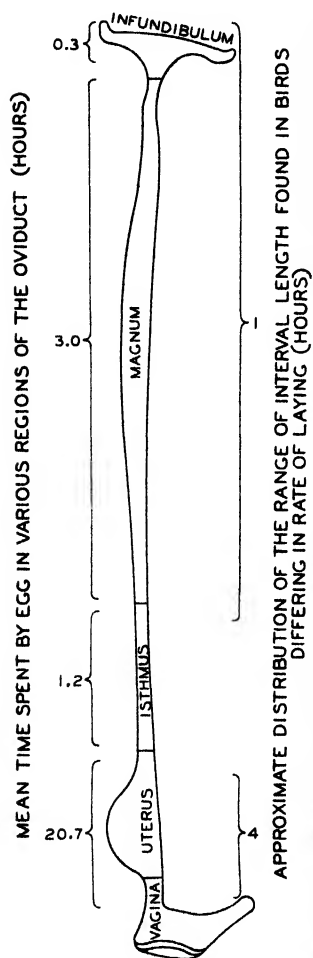


FIGURE 2.—Regions of the oviduct where the egg spends the additional time in birds with exceptionally long intervals (low intensity). The distribution of the excess time in the oviduct is shown in relation to the time required for egg formation in the different regions of the oviduct.

time required to reach the isthmus has any significance in relation to interval length. In the previous work by the authors⁶ the time required for passage of the egg through the magnum in anesthetized birds was very uniform. In the earlier studies there was found to be much more variability in the time between laying and ovulation. The means given in the first line in table 1 include the time between laying of the one egg and the ovulation of the next as well as the time required to traverse the length of the magnum. There was no consistent agreement between the increases in length of the interval and the length of the period between the previous laying and the entry of the next egg into the isthmus. There was, however, a difference of 1 hour in time required for the egg to reach the isthmus in birds with the longest and shortest intervals. If this 1-hour difference is accepted as being significant, it would seem that the lengthening of the interval is uniformly distributed throughout the oviduct. Twenty percent of the total variability of the interval is accounted for in the period required for ovulation and traversing the magnum by the egg and approximately that same percentage of the whole period of egg formation has elapsed when the egg reaches this point (posterior end of magnum). So, although the amount of the variation in length of interval accounted for in the magnum is small, it is as large as

might be expected for a section of the oviduct in which the egg spends so little time. This relationship is indicated more clearly in figure 2. It may be questioned whether the 1-hour variation in time spent in magnum has any significance when the observations were made at 2-hour intervals.

In an earlier study absolute or relative variations in time spent in the magnum, or time required for ovulation, could not be corre-

⁶ WARREN, D. C., and SCOTT, H. M. See footnote 5, first reference.

lated with differences in mean interval length. If the variation found here is considered as significant it must be accounted for either as a delay in ovulation or a retardation of the rate of passage through the magnum in birds with longer intervals. Since so little variation was found in the rate of passage through the magnum and since there was considerable variability in time required for ovulation, the writers are inclined to designate the latter as the probable source of the lengthening of the interval in this region. The maximum variation in time between laying of the previous egg and the next ovulation in anesthetized birds was a little over 1 hour.

The data on time spent in the uterus by eggs having varying interval lengths are given in the second line of table 1. This period was measured by the time intervening between the egg reaching a plump stage and its being laid. The egg probably requires about 2 hours to become plump after entering the uterus, so this measurement is fairly accurate for estimating the period spent by the forming egg in the uterus. When the egg first enters the uterus the membranes are rather loose fitting and fluids then pass through them causing the egg to take on the plump state before any calcium deposition can be detected. The data in the third line of table 1 are the intervals between the time when the presence of shell material could first be detected and the laying of the egg. The shell formation can probably be detected in about 5 hours after the egg enters the uterus. The time of detection of either of these stages (plump or shell) may be used as a point from which to calculate the relative time spent in the uterus by eggs of different interval lengths.

It is shown that the period spent in the uterus, as measured by the time elapsing between the plump membranous stage (or the early shell formation stage) and laying, varies directly with the interval length. With each increase of an hour in interval length there is a corresponding, though not always equal, increase in hours spent in the uterus. For the 5 hours' difference in interval length, 3.6 hours are accounted for in the extremes from the plump stage to laying and 4.1 hours in the extremes between the first indication of shell formation and laying. In the former case 1.4 hours and in the latter 0.9 hour of the variation in interval are unaccounted for in the variations of time spent in the uterus. Since extremes of interval length are included perhaps one should not expect the means of time spent in the uterus to account for these extremes even though the uterus is the section of the oviduct where the variations in interval length occur. It can at least be stated that most of the variability in the absolute length of the interval may be accounted for by differences in time that the egg remains in the uterine region. It should be kept in mind that only those differences in interval are here considered which are found in birds having clutches of two or more eggs in size. Birds with 1 egg clutches were not included because of the impossibility of accurately calculating interval length. Also the long interval occurring between clutches is excluded.

It should be emphasized that normally approximately 82 percent of the interval is accounted for by the time the egg remains in the uterus. So if the difference in time of egg formation between long- and short-interval birds is uniformly distributed over the entire process of egg formation much of the difference would be accounted for

in the uterine stage. In accounting for the differences in interval length the time spent in the isthmus has not been discussed. The formation time in this region is so short and methods of locating the egg in this section of the oviduct so crude that it was not considered feasible to make comparisons in this region. It is also true that the differences encountered in interval length had all been accounted for in the magnum and uterus.

OVULATION DELAY AND CLUTCH

The foregoing study has accounted for much of the difference in interval length which is an important factor in rate of egg production. There remains a second factor which contributes to intensity or rate of laying. This is the length of the period between clutches. The occurrence of a break between clutches is due to a delay of a few hours in ovulation. For successive eggs in a clutch ovulation occurs practically immediately following the expulsion of the preceding egg. The length of the period interrupting the regular sequence of ovulation was measured by the period intervening between the detection of the presence in the oviduct of the first egg of the clutch and the laying of the last egg of the previous clutch. This period includes the time spent in the magnum by the first egg of the clutch as well as the period of delay in ovulation. These two factors cannot be segregated one from the other but it has already been shown that the former varies but little. Therefore, this interval may be used as a measurement of the variability in delay of ovulation. Since the length of the clutch is conditioned largely by the interval length, clutch size may be used for grouping the birds as to their interval length. They were placed in six arbitrary grades of increasing clutch size and the respective periods of delay of ovulation were 22.9, 22.0, 19.9, 19.0, 17.2, and 16.5 hours. It is evident that the birds with small clutches, long intervals, and low intensity, also have the longer delays in ovulation. Thus, the poor production of low-intensity birds is due not only to the longer period of egg formation but also to a longer delay in ovulation between clutches where such delays occur. From autopsies it was frequently noted that very low-rate birds showed evidence of ova having been released in the abdominal cavity. In such birds the rate of laying is probably not an accurate measure of rate of ovulation.

SUMMARY

It was shown that much of the difference in the absolute length of interval between eggs, which is the major factor in controlling rate of laying, is due to variations in time that the egg remains in the uterus. The time spent in the magnum (albumen-secreting section) plus the time between the previous laying and ovulation were only slightly variable and could account for only a small part of the absolute variation found in interval length. These conclusions were arrived at from data secured by tracing the forming egg while in the oviduct of living birds.

In low-intensity birds there was also a lengthening of the delay in ovulation which occurs in the case of the first egg of a clutch. Thus, low intensity of production is due to both a longer period for passage of the egg through the oviduct and to a longer pause between clutches.

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A CYTOLOGICAL STUDY OF THE RESISTANCE OF APPLE VARIETIES TO *GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE*¹

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INTRODUCTION

A cytological study of the succession of pathologic events following the infection of susceptible and resistant host plants by the rust fungi affords a means of comparing the parasitic behavior of these organisms and contributing to the understanding of the mechanism of the resistance against their attack.

Since Biffen (11)³ showed that the resistance of wheat to leaf rust, caused by *Puccinia triticina*, was a heritable character independent of the genetical factors responsible for anatomical features and Ward (34, 35, 36) stressed the concept of physiological resistance, many cytological studies have been made upon the rust diseases. The value of this avenue of approach is illustrated by the work upon the cereal rusts by Ward (34), Stakman (33), Allen (1, 2, 3, 4, 5, 6), Ruttle and Fraser (32), and others, who have presented a critical analysis of problems pertaining to the nature of rust resistance. The earlier work is reviewed by Allen (1), Rice (31), and Zimmermann (39) and need not be discussed here.

In accord with the behavior of rust fungi in general, the apple rust fungus, *Gymnosporangium juniperi-virginianae* Schw., shows a wide range in pathogenicity upon the foliage of the different varieties of its aecal host, the apple, *Pyrus malus* L. The degree of severity of the disease does not afford a rigid classification because many gradations exist between extreme susceptibility and apparent immunity. On susceptible varieties, the development of the fungus culminates in the production of the aecal stage. On the other hand, there are varieties upon which no macroscopic signs of infection appear. Between these two extremes lie those resistant varieties upon which the fungus becomes established but does not develop very far beyond the incipient stages. In some cases, various types of flecking are produced, while in others the lesions may be well defined, and minute or aberrant pycnia may appear.

Previous studies of the nature of rust resistance in apple varieties have been confined to secondary considerations supplementary to the broader epidemiological and pathological aspects of the disease. From field observations of the occurrence and severity of infection upon different varieties, Reed and Crabill (30) believed resistance to

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² Grateful acknowledgments are made to Dr. E. M. Gilbert for his kind criticism and advice and especially to Dr. G. W. Kelt for his helpful direction throughout these investigations and in the preparation of the manuscript. Thanks are given to Eugene Herring for assistance in the preparation of photomicrographs.

³ Reference is made by number (italic) to Literature Cited, p. 594.

be of two types: (1) The prevention of infection, due to the mechanical, physical, or chemical nature of the epidermis, and (2) the incompatibility of the host and parasite after infection had occurred, resulting in the production of an aberrant lesion. They described briefly the pathological histology of apple rust infections in a susceptible host and made microscopic examinations of infections in the leaves of Arkansas, a resistant variety upon which abnormal lesions occurred. However, the sequence of events in the development of these infections and the cytological details involved were not reported.

Leaves of susceptible varieties become resistant with age. Giddings and Berg (20) and Reed and Crabill (30) attached much significance to this phenomenon as a factor in the regulation of the severity of the disease. They discussed the nature of resistance in mature leaves but did not give factual evidence to explain it. Miller (27) observed the occurrence of normal infections upon mature leaves of susceptible varieties that had been wounded mechanically prior to inoculation. This led to the interpretation that the resistance shown by mature leaves had a morphological basis.

The sporidial germ tubes of *G. juniperi-virginianae* commonly infect apple leaves through the ventral epidermis. Reports of dorsal-surface infections are at variance. Coons (14) implied that infection of the lower epidermis occurred but stated, however, that upper-surface inoculations were more successful. The experiments of Giddings and Berg (20) showed that no infection followed inoculation of the lower epidermis. On the other hand, Weimer (38) reported that infection could take place upon either surface. Dorsal surface infection, however, had no effect upon the normal orientation of the pycnia and aecia. In the literature dealing with apple rust, further information with a bearing upon the nature of resistance has not been found.

From the above accounts, it is apparent that little is known concerning the parasitic behavior of the apple rust fungus or the nature of apple rust resistance. In view of these problems, a detailed cytological study of rust infections upon four apple varieties, each differing in its reaction to the fungus, was undertaken. The results of these studies are presented in this paper.

MATERIALS AND METHODS

Four varieties of apples were used in these studies as follows: Wealthy, susceptible; Yellow Transparent, moderately resistant; Fameuse, resistant; and Baldwin, very resistant. Two-year-old nursery trees were placed in galvanized-iron containers in about 10 kg of soil adjusted and held at 70 percent of the maximum water-holding capacity. After being rooted in a cool basement, they were placed on the greenhouse bench. One shoot was allowed to develop on each tree. When the shoots were about 18 inches long and showed 14 to 20 leaves, they were inoculated.

Rust material for inoculation was collected near Madison, Wis. Branches of red cedar (*Juniperus virginiana* L.) bearing abundant rust galls with extended telial sori were brought into the laboratory and the cut ends of these twigs were kept in water until the material was used.

Several methods of inoculation were tried with varying results. The following method proved most useful and was employed through-

out: The rust galls were moistened in a spray of water for 4 hours, after which the telial sori were well gelatinized. A few hours later, when sporidial discharge was taking place rapidly, the galls were suspended in a glass lamp chimney which was held above the leaves to be inoculated in such a way that the sporidia would fall upon the upper surfaces of the leaves. One lot of leaves of the Wealthy variety was inoculated on the lower surface. Each shoot was exposed for a 15-minute period. The inoculated trees then were placed in a moist chamber, described by Keitt and Jones (21), at 14° C. for 36 hours, after which they were returned to the greenhouse bench.

Question may arise regarding the purity of the rust culture used. Recently, Bliss (12) reported the existence of physiologic forms in *G. juniperi-virginianae* collected from widely separated localities. The writer believes, however, that only one physiologic form was used in these studies because the material was collected from cedar trees in an isolated group and inoculation experiments upon many apple varieties with similar cultures during the past 3 years have shown uniform pathogenicity.

After inoculation, collections were made at daily intervals for the first week and at longer intervals thereafter for the next 2 weeks. One earlier collection was made after a 14-hour period. At the time of inoculation, a bit of string was tied about the petiole of the youngest leaf in order to facilitate identification of the inoculated leaves after further growth had occurred. Material always was taken from the fourth or fifth leaf below the "stringed" leaf to insure uniformity. One lot of Wealthy leaves, which had attained approximately full size, and another group about 3 weeks older were collected in an attempt to gain evidence pertaining to the relationship of maturity to resistance. Fixations were made in formal-chrom-acetic mixture, in Flemming's medium and weak mixtures, and in the formal-acetic-alcohol fixative. Flemming's weak fluid was the most useful for young material, whereas the formal-chrom-acetic mixture was best for the later stages. The butyl alcohol method of Zirkle (40) was used for dehydration and embedding in paraffin. Sections were cut 5 μ and 6 μ thick. A modification of Flemming's triple stain, consisting of safranin, gentian-violet, and fast green, gave satisfactory results.

A modification of the cleared-leaf method of Peace (29) was well adapted to a superficial study of sporidial germination and the early stages of penetration because it afforded a means of observing the germinating sporidia in toto upon relatively large areas of leaf surface. Bits of leaf were collected at 7, 14, and 24 hours after inoculation, fixed in acetic-alcohol mixture, cleared in chloral hydrate, and stained with acid fuchsin in lactophenol.

MACROSCOPIC OBSERVATIONS ON LIVING LEAVES

On the immature leaves of Wealthy, inoculated upon the ventral surface, pale yellow spots 1 mm in diameter appeared on the seventh day. The lesions increased in size to about 2 mm during the next 2 days and became orange in color. Pycnia appeared on the thirteenth day and pycnial exudation occurred on the fifteenth day. The heaviest infection appeared on the fourth, fifth, and sixth leaves below the stringed leaf. At the time of inoculation, these leaves were rapidly expanding and had not reached full size. No infection was

found below the tenth leaf, indicating that the older, matured leaves had become resistant. Wealthy leaves, inoculated upon the dorsal surface only, showed an occasional spot appearing on the twelfth to the fourteenth day. These lesions developed in the usual manner. The prolonged incubation period suggests that the parasite may have had some difficulty in becoming established.

On the leaves of Yellow Transparent infection appeared on the ninth day as indistinct pale yellow spots about one-quarter millimeter in diameter. A few days later the infected leaves showed a pale yellow mottling with few well-defined lesions. On the eighteenth day an occasional pycnium could be seen in the regions where the lesions were more clearly outlined. These pycnia were minute and failed to rupture the epidermis or exude.

On the leaves of Fameuse, the first signs of infection appeared on the twelfth day as tiny flecks which were barely visible. No further development was evident.

On the leaves of Baldwin, no macroscopic signs of infection showed throughout the 3-week period the trees were under observation. These external appearances indicated complete resistance.

EARLY STAGES OF INFECTION

CLEARED-LEAF STUDY

The phenomena of sporidial germination and of the early stages of penetration, as observed on cleared leaves, appeared to be similar on all varieties. Counts made on germination of sporidia, formation of appressoria and the appearance of infection hyphae showed insignificant differences (table 1).

It is not probable that these data are vitiated to any appreciable extent by the removal of some of the sporidia by the reagents used in clearing and staining the leaves. Previous experience of the writer indicates that, when the sporidia become lodged upon the leaf surface, they cling tenaciously.

After the 7-hour period, nearly all of the sporidia had germinated. The germ tubes in no case exceeded in length the diameter of the sporidium. There was no indication that the tips of the germ tubes were attached to the leaf surface.

TABLE 1.—Data on germination of 100 sporidia, formation of appressoria, and development of infection hyphae on four apple varieties

Variety	Age of leaf	Surface inoculated	Percentage development after stated periods								
			7 hours			14 hours			24 hours		
			Germi-nated	Ap-pres-soria	Infec-tion hy-phae	Germi-nated	Ap-pres-soria	Infec-tion hy-phae	Germi-nated	Ap-pres-soria	Infec-tion hy-phae
Wealthy	Young	Ventral	91	0	0	98	89	8	97	97	97
	do.	Dorsal	98	0	0	95	91	21	97	97	97
	Old	Ventral	88	0	0	99	83	22	96	96	96
Yellow transpar-ent.	do.	Dorsal	92	0	0	96	89	10	100	100	100
	Young	Ventral	94	0	0	95	84	11	99	99	99
Fameuse	do.	do.	95	0	0	100	92	15	97	97	97
Baldwin	do.	do.	93	0	0	95	86	5	96	96	96

After the 14-hour period, the process was distinctly further advanced. Most of the germ tubes had formed appressoria, the term "appressorium" being used in a broad sense throughout to describe the swelling of the tip of the sporidial germ tube adherent to the leaf surface. The germ tubes were seldom longer than the diameter of the sporidium and, in many cases, they were so short that the appressoria appeared to be attached directly to the sporidia. The appressoria were not well defined. They appeared only as swellings of the germ-tube tips. A few of these appressoria showed a minute porelike

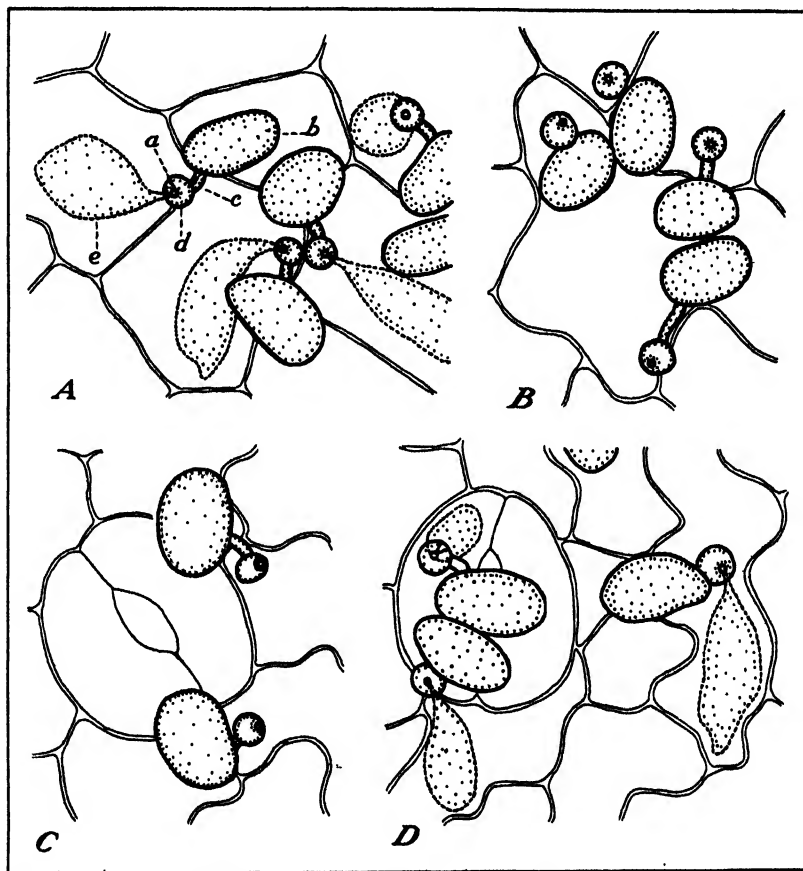


FIGURE 1.—Camera-lucida sketches of sporidial germination and host penetration from cleared leaves examined in toto: A, ventral surface of young Wealthy leaf, pore of infection hypha (a), sporidium (b), germ tube (c), appressorium (d), primary hypha in epidermal cell (e); B, ventral surface of old Wealthy leaf; C, dorsal surface of old Wealthy leaf; D, dorsal surface of young Wealthy leaf. Approximately $\times 775$.

structure, about 1μ to 2μ in diameter, in the wall in contact with the leaf. This phenomenon suggested the development of an infection hypha. There was, however, no apparent change of the underlying host tissues to indicate that the fungus had entered.

After the 24-hour period, all of the germ tubes were firmly attached to the leaf surface and characteristic pores were clearly visible in all of the appressoria. In some cases the appearance of a large primary

hypha in the underlying epidermal cell indicated that penetration had occurred, but this manifestation was too indistinct to be relied

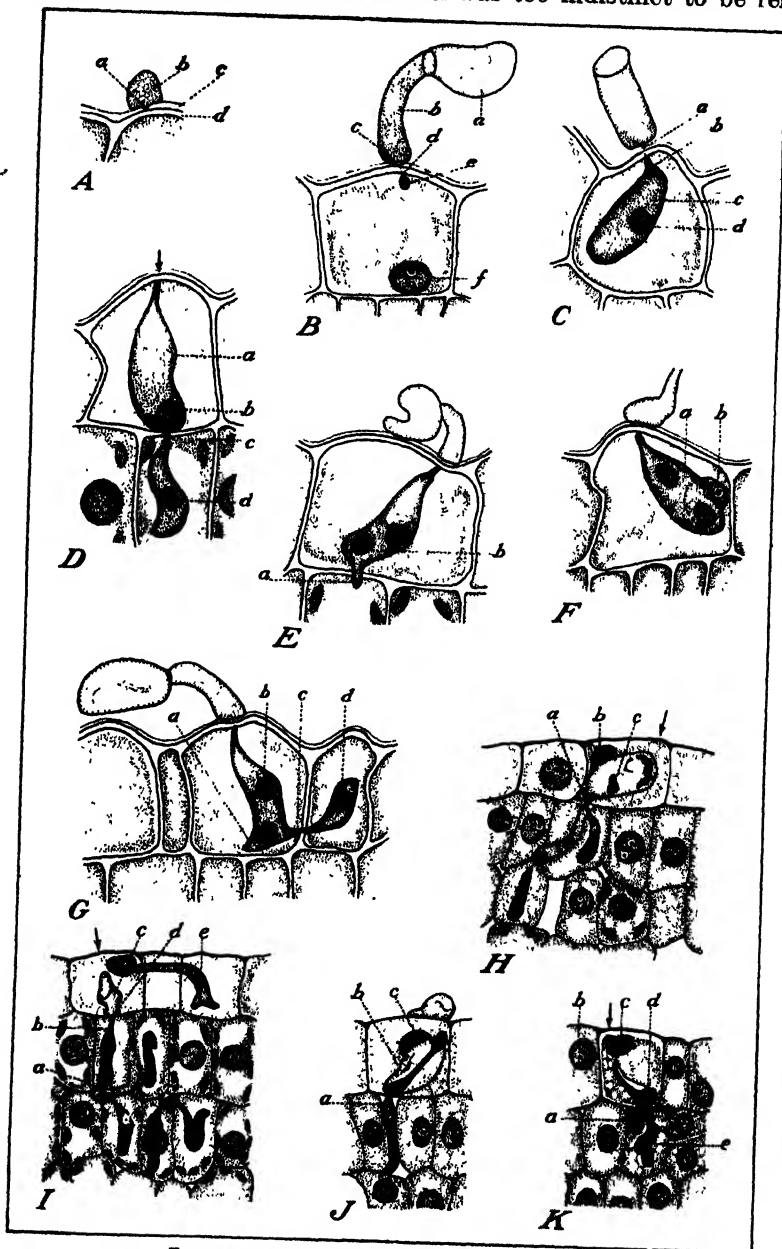


FIGURE 2.—For explanatory legend see opposite page.

upon as an absolute criterion. The appearance of the 24-hour stage of development upon both upper and lower surfaces of young and old Wealthy leaves is shown in figure 1.

This study was not carried further because a technic was not found which would satisfactorily stain the fungus within the leaf.

PHENOMENON OF PENETRATION

The fungus entered the immature leaves of all four varieties and in all cases the process was essentially the same. The germ tubes emerged from the sides of the sporidia and curved obtusely, sometimes almost at right angles, down to the leaf surface, where the germ-tube tips swelled slightly into small appressoria which were firmly attached to the cuticle (fig. 2, *E, G*). Waterhouse (37), working with *Puccinia graminis* on the barberry, reported that germ tubes were not always formed. In case they were not, a short beaklike infection hypha was put out from one end of the sporidium itself and dented the cuticle. Allen (7), also working with *P. graminis*, observed that the sporidia directly produced a beak which pierced the cuticle and outer epidermal wall. After 14 hours, the lower wall of the appressorium of the apple rust fungus showed a short protrusion partly penetrating the cuticle (fig. 2, *A*). At that time, the sporidium was nearly evacuated and the germ tube was filled with dense protoplasm. Nuclei could not be seen.

The earliest stage of penetration is shown in a 24-hour infection in figure 2, *B*. The minute pore is shown at *c* and from it the infection hypha (*d*) can be followed part way through the upper wall of the epidermal cell to the young primary hypha (*e*) in the cavity of the cell. The host nucleus remains lying in the bottom of the cell at *f*. In no case could the infection hypha be traced all the way through the cuticle and the cell wall, there being a short space about half-way through the barrier, where it could not be seen. This suggests that it was ultramicroscopic or was not sufficiently stained in that portion to be visible. The facts that there was no apparent alteration of the cuticle or epidermal cell wall at the point of entry and that the cuticle was often indented indicate mechanical pressure as the principal factor involved in penetration. This is in agreement with Waterhouse (37). Weimer (38) described and figured one case where a sporidial germ tube of *G. juniperi-virginianae* penetrated

EXPLANATORY LEGEND FOR FIGURE 2

- A*.—Early stage of penetration on Wealthy, 14 hours after inoculation. The beak (*a*) of the appressorium (*b*) is indenting the cuticle (*c*) but has not penetrated the epidermal cell wall (*d*). $\times 1,070$.
- B*.—Penetration on Wealthy, 24 hours after inoculation. Empty sporidium (*a*), germ tube (*b*), penetration pore (*c*), infection hypha (*d*), young primary hypha (*e*), and nucleus of epidermal cell (*f*). $\times 1,070$.
- C*.—Early stage of infection on Wealthy, 24 hours after inoculation. The primary hypha (*c*) contains one nucleus (*d*) and remains attached to the infection hypha (*e*) by a slender neck (*b*). $\times 1,070$.
- D*.—One-day-old infection on Wealthy. Arrow indicates point of entry. Primary hypha (*a*) is slightly evacuated and contains one nucleus (*b*); secondary branch has penetrated palisade cell at *c* and contains one nucleus (*d*). $\times 1,070$.
- E*.—One-day-old infection on Wealthy. Young secondary branch (*a*) has penetrated palisade cell and one of the nuclei (*b*) is ready to pass into it. $\times 1,070$.
- F*.—One-day-old infection on Wealthy. The primary hypha (*a*) is binucleate; epidermal cell nucleus in attendance at *b*. $\times 1,070$.
- G*.—One-day-old infection on Wealthy. The primary hypha (*b*) sent a branch (*d*) into an adjoining epidermal cell at *c*; another branch appears as a beak (*a*) and a nucleus is ready to pass into it. $\times 1,070$.
- H*.—Two-day-old infection on Yellow Transparent. An arrow indicates the point of penetration. The nucleus (*c*) of the primary hypha is dead; a secondary branch entered the palisade intercellularly at *a*; the epidermal cell nucleus (*b*) is degenerating. $\times 700$.
- I*.—Two-day-old infection on Wealthy. An arrow indicates the point of penetration. The primary hypha (*c*) sent a branch (*e*) into an adjoining epidermal cell and another branch (*b*) into a palisade cell at *d*; mycelium becoming intercellular at *a*. $\times 700$.
- J*.—Two-day-old infection on Fameuse. Primary hypha (*b*) has collapsed; host cell nucleus (*c*) is degenerating; intercellular branch (*a*) is developing feebly. $\times 700$.
- K*.—Two-day-old infection on Baldwin. An arrow indicates the point of penetration. Primary hypha (*d*) and intracellular secondary branch (*e*) are dead; host nuclei (*a* and *c*) are degenerating; host nucleus (*b*) is slightly enlarged. $\times 700$.

the epidermis of a Wealthy apple leaf and passed about two-thirds of the distance through the epidermal cell 7 hours after inoculation. This must have been an unusual case. Examination of several hundred germinating sporidia by cleared leaf and cytological methods failed to show that any had penetrated the epidermis completely after 14 hours.

Other 24-hour infections showed stages considerably advanced over the one described above. The instance in figure 2, *C*, shows that the primary hypha (*c*) has enlarged into a turgid, saccate body which remained joined to the infection hypha (*a*) by a slender neck (*b*). The hypha contains one nucleus (*d*) and is filled with dense cytoplasm. The nucleus is probably the one originally contained in the sporidium and has not yet divided. Allen (9) reported that in *Puccinia coronata* the sporidial nucleus divided before penetration and that the primary hypha, therefore, contained two nuclei. Two nuclei were also found in the primary hypha of *P. graminis* (7) but whether or not division took place before entry was not investigated.

In these preparations of the younger stages of infection and of the older ones as well, the fixations obtained did not afford a clear interpretation of the relationship between the membranes of the host cell and the fungus. It was impossible to determine whether the host-cell cytoplasm was invaginated by the primary hypha or haustoria or actually penetrated by them. The views of other workers on this point are reviewed by Rice (31).

During the process of penetration and the expansion of the primary hypha of the apple rust fungus, there was no apparent change in the staining reaction of the parasitized epidermal cell. Further development, however, depended upon the degree of resistance of the host. Consequently, the reactions in each variety are reported separately.

DEVELOPMENT OF INFECTION ON THE FOUR VARIETIES

WEALTHY

The establishment of the fungus in the leaf tissues of Wealthy presented a picture of almost complete compatibility between the two organisms. This condition existed until pycnial formation when distinct changes accompanied the fruiting activities of the parasite.

The behavior of the young primary hyphae in the epidermal cells was simple and afforded trustworthy interpretation. Soon after the primary hypha attained full size, the nucleus divided (fig. 2, *F*). These nuclei were similar in appearance to the parent nucleus. No septa appeared in the primary hypha and the structure remained simple throughout its existence. In *Puccinia graminis* (7), *P. coronata* (9), and *P. triticea* (8) a more complicated process, similar in all cases, was reported by Allen. Nuclear and cell division occurred until from 3 to 6 cells were produced. The primary hypha increased in length and branches of secondary hyphae arose from each cell. In *Melampsora lini* (10), the primary hypha consisted of several uninucleate cells, irregularly branched. Palisade penetration occurred at many points.

The number of secondary hyphal branches of the apple rust fungus seemed to be dependent upon the behavior of the first one formed. In figure 2, *G*, a branch (*d*) has penetrated into an adjoining epidermal

cell at *c*, and contains one of the daughter nuclei of the first division. Another nuclear division has occurred in the primary hypha and one of the nuclei is ready to pass into a new branch which appears as a beak at *a*.

The formation of only one secondary branch, penetrating the palisade tissues directly, was the most common occurrence. In figure 2, *D*, a branch (*d*) has entered the palisade cell beneath and a nucleus has entered, leaving one (*b*) in the primary hypha. The primary hypha has the appearance of being evacuated and the branch is filled with dense protoplasm. In *E*, the branch appears as a peg-like outgrowth (*a*) which is penetrating into the underlying palisade cell and a nucleus (*b*) is ready to pass into it.

Palisade penetration occurred without any apparent retardation of apical growth. There was no constriction of the hypha at the point of cell-wall penetration and no visible alteration of either the cellulose or middle lamellar portions of the walls. Leach (23) working with *Colletotrichum lindemuthianum*, observed that the primary hypha bent, and that the advancing tip swelled, due to the retardation of apical growth during cell-wall penetration. He also stated that the hole made through the cell wall was very small and that the mycelium was constricted at that point. Allen reported that the opening in a cell wall through which a branch of the secondary hypha of *Puccinia graminis* (?) passed was too small to be seen, but that in *P. triticea* (8) it was large.

Similar to *Puccinia graminis* (?), the early development of the secondary hyphae of *Gymnosporangium juniperi-virginianae* depended upon whether or not they entered palisade cells or intercellular spaces. If they were intercellular, the hyphae immediately expanded into characteristic strands of mycelium. If they were intracellular, they assumed the appearance of large haustoria, each connected to the point of entry by a slender neck.

A 2-day-old infection is shown in figure 2, *I*. An arrow points to the place of entry. The primary hypha (*c*), which was partly lost in sectioning, is slightly evacuated. One branch (*e*) (the connection was lost in sectioning) has penetrated the adjoining epidermal cell. This secondary hypha contains one large nucleus and is forming two new branches at the distal end. Palisade penetration occurred intracellularly at *d*. The penetrating hypha (*b*), is somewhat swollen, is unbranched, and consists of only one cell. It has passed entirely through the cell and emerged into an intercellular space at *a*. From here on, the mycelium is regularly intercellular with the formation of characteristic uninucleate haustoria.

While still in the palisade region, the hyphae exhibited difficulty in passing through the compact tissues. The hyphal tips, viewed longitudinally, appeared as wedges prying the cell walls apart. The fact that there was no splitting apart of the walls in advance of the hyphal tips precluded the view that the fungus might have some solvent action upon the middle lamella. It seemed that considerable pressure would have to be exerted to force a passage mechanically, but that is probably what actually occurred. Allen (9) made similar observations upon *Puccinia coronata* where the early development of the fungus regularly takes place in the compact subepidermal region.

The haustoria entered the cells from the intercellular hyphae without the formation of attachment organs. The only stimulus neces-

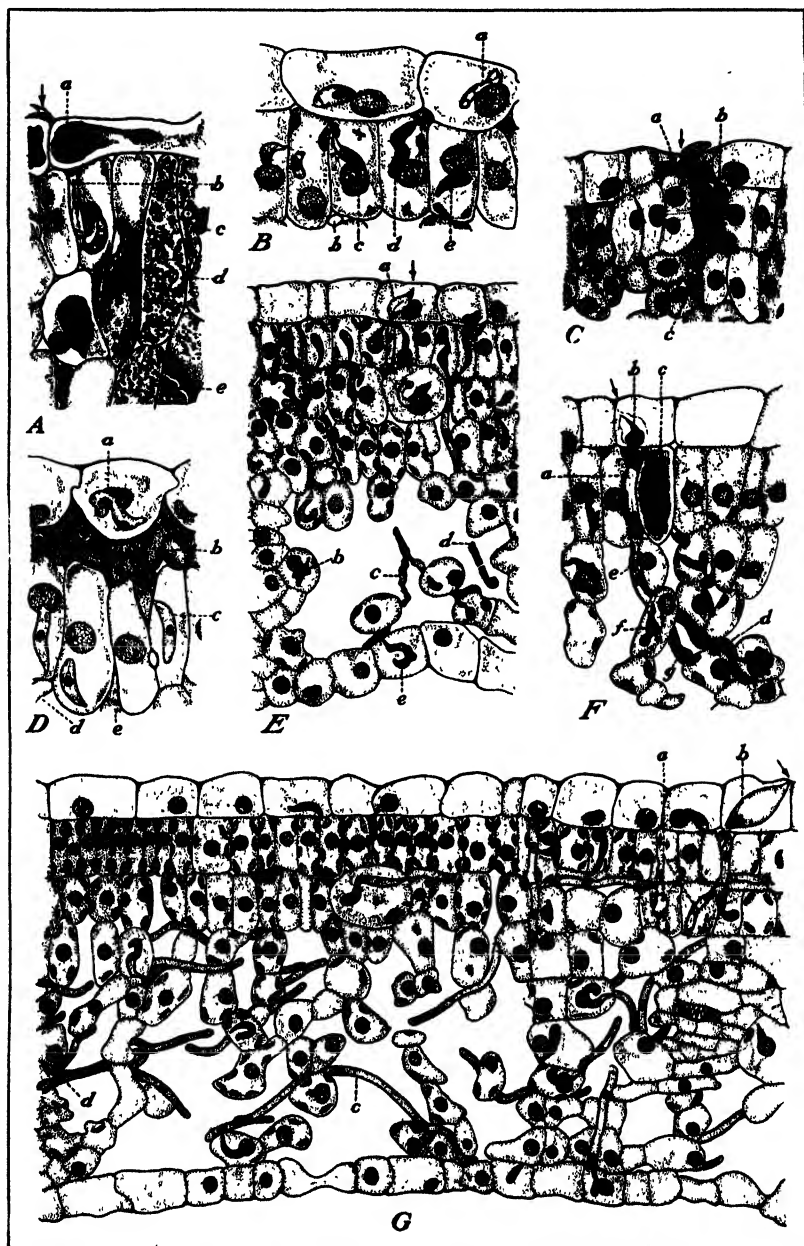


FIGURE 3.—For explanatory legend see opposite page.

sary for haustorium formation seemed to be close contact of the tip or side wall of a hypha with the host-cell wall. A nucleus was usually found in the hypha near the point of haustorium formation and the

haustoria were regularly uninucleate. It seems reasonable to assume, therefore, that a nuclear division took place in the hypha and that one of the daughter nuclei passed into the haustorium, although the process was not observed. The pore of entry was about 2μ to 3μ in diameter and could be seen clearly. There was no apparent change in the cell wall at that point. The main body of the haustorium was connected to the intercellular hypha by a slender neck. In all of the younger infections the haustoria were one-celled and uninucleate, without any branching. The haustoria generally were filled more densely with protoplasm than the intercellular hyphae and took up the violet of the triple stain heavily.

Growth of the mycelium was accelerated greatly after the second day. A median section of a 4-day-old lesion is shown in figure 3, *G*. An arrow indicates the point of entry, and the primary hypha, turgid in appearance and containing one nucleus, is shown at *b*. Intercellular penetration of the palisade occurred and the secondary hypha immediately branched and ramified the local palisade region to a limited extent. Simple, uninucleate haustoria occupy most of the palisade cells in the region of penetration and slight depletion is evident in the reduction in the number of plastids. Two cells at *a* show disorganization of their protoplasts. The host nuclei in the parasitized cells were usually in contact with the haustoria but only because of the vigorous expansion of the haustoria. There was no apparent movement of the nuclei in response to the presence of the fungus. Haustoria were not seen which encircled the nucleus or changed its shape due to contact or the application of pressure against it. In fact, all of the host nuclei retained their normal structure and staining reaction.

After gaining access to the large intercellular spaces of the spongy parenchyma region, the fungus spread rapidly. Long hyphae appear as runners (fig. 3, *G*, *c*, *d*) which have already spread a distance of about 200μ from the point of entry. These runners are sparsely branched, contain dense protoplasts, and have widely separated septa. The cells are uninucleate. Occasional parenchyma and lower epidermal cells along their path contain characteristic haustoria which, no doubt, help to supply food for this rapid growth.

Spread of the mycelium was much less rapid in the palisade than in the spongy parenchyma. Haustoria, however, were more numerous

EXPLANATORY LEGEND FOR FIGURE 3

A.—Eight-day-old infection on Fameuse. An arrow points to the remains of the appressorium. Epidermal cell (*a*) contains disintegrated primary hypha and collapsed host-cell contents; intercellular hypha (*b*) is vacuolate; degenerating host-cell protoplast invaginated by dead haustorium (*e*); haustorium at *d* empty and one at *c* killed back from tip. $\times 650$.

B.—Seven-day-old infection on Yellow Transparent. Dead haustoria (*d* and *e*) occupying living cells, normal haustorium (*c*), empty haustorium (*a*), evacuated hypha (*b*). $\times 650$.

C.—Four-day-old infection on Baldwin. An arrow indicates the point of penetration. The epidermal cell of entry has collapsed and contains the remains of the dead primary hypha. Haustorium (*a*) in a living cell died before it fully expanded; palisade cells (*b*) are dead; host cell (*c*) disintegrating. $\times 440$.

D.—Pycnial initiation on Wealthy, 9 days after inoculation. Mass of hyphae (*b*) in subepidermal region; haustoria (*a* and *c*) and intercellular hyphae (*d* and *e*) becoming evacuated; host cells impoverished. $\times 650$.

E.—Four-day-old infection on Yellow Transparent. An arrow indicates the point of penetration. Primary hypha (*a*) partly collapsed; haustorium (*b*) and hypha (*d*) are dead; hypha at *c* degenerating; normal haustorium in lower epidermal cell at *e*. $\times 440$.

F.—Four-day-old infection on Fameuse. An arrow indicates the point of penetration. Primary hypha (*b*) dead; intercellular branch (*a* and *c*) degenerating; host cell (*c*) contains the remains of a dead haustorium and of its own collapsed protoplast; dead haustorium (*f*) in a living cell; advancing hyphae (*d* and *g*) densely filled with protoplasm. $\times 440$.

G.—Four-day-old infection on Wealthy. An arrow indicates the point of penetration. Primary hypha (*b*) uninucleate and turgid; vacuolate haustoria (*c*) occupy cells with broken down protoplasts; stolonlike branches in spongy parenchyma region at *e* and *d*. $\times 440$.

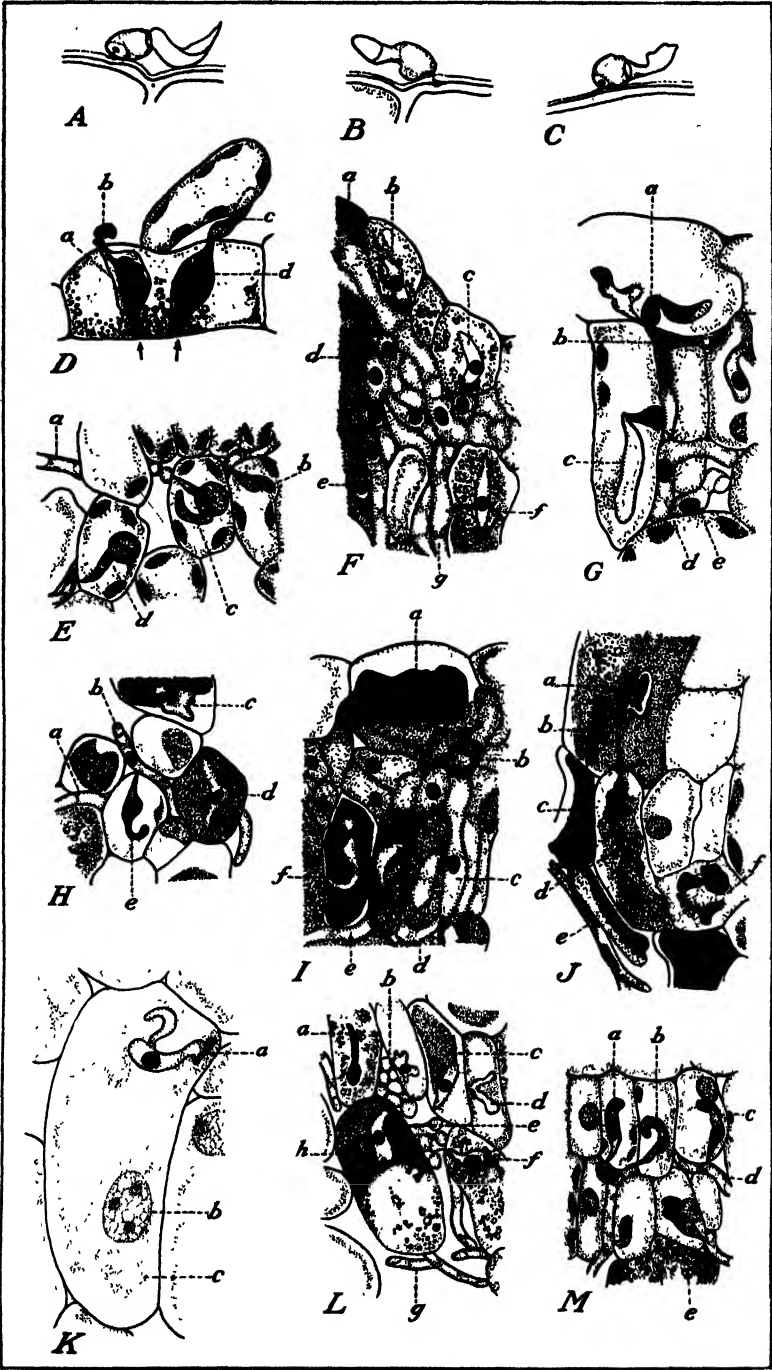


FIGURE 4.—For explanatory legend see opposite page.

and in a few cases appeared in the upper epidermal cells. This is not in agreement with Reed and Crabill (30) who reported that they did not find haustoria in the epidermal cells of apple rust infections.

From the fourth to the tenth day, there was very little change in host-parasite relations. The lesions continued to spread in all directions, especially in the spongy parenchyma region. In the older parts of a 9-day-old infection (pl. 1, *A*) nearly every host cell contained an haustorium. Depletion of the cytoplasm of these cells was still slight, although many of the plastids had disappeared. The margin of the lesion appeared similar to the margin of the 4-day-old lesion. Near the center of the infection (fig. 3, *D*), hyphae had begun to accumulate in the subepidermal region preparatory to pycnial formation. These hyphae were filled with dense cytoplasm (*b*) whereas the haustoria (*a* and *c*) and other vegetative hyphae (*d* and *e*) appeared to be nearly empty.

After the pycnia had ruptured the epidermis, the activity of the host and of the parasite showed decidedly different trends. A photomicrograph of a 15-day-old infection (pl. 1, *C*) shows the general character and location of the reactions.

In the spongy parenchyma region below the pycnia, hypertrophy of the cells caused the obliteration of the large intercellular spaces and the collapse of a large portion of the lower epidermis. A representative cell from this region is shown in figure 4, *K*. It is approximately four times normal size and contains a uninucleate, branched haustorium at *a*, which is almost empty. The host nucleus (*b*) is approximately twice the normal size and is stained very faintly, giving the impression that the reticulum was loosening. The lumen of the cell otherwise appears empty except for a thin strand of cytoplasm (*c*). The intercellular hyphae appeared to be empty except for their nuclei.

EXPLANATORY LEGEND FOR FIGURE 4

A.—Penetration failure upon old Wealthy leaf, 3 days after inoculation. Cuticle indented and appressorium partly raised caused by the pressure of the infection hypha which has failed to penetrate. $\times 850$.

B.—Penetration failure upon old Wealthy leaf, 3 days after inoculation. Infection hypha has penetrated the cuticle but failed to puncture the epidermal cell wall. $\times 850$.

C.—Penetration failure upon old Wealthy leaf, 3 days after inoculation. Infection hypha penetrated the cuticle and flattened out against the epidermal cell wall; the appressorium is slightly raised. $\times 850$.

D.—Dorsal surface of young Wealthy leaf, 5 days after inoculation. Arrows indicate the point of penetration in two separate infections. Primary hyphae (*a* and *d*) are dead and their secondary branches (*b* and *c*), after having emerged into large intercellular spaces, have also succumbed. $\times 850$.

E.—Tissues from the marginal region of a 15-day-old infection on Wealthy. Normal haustoria (*b*, *c*, and *d*) occupying living cells; vacuolate hypha (*a*). $\times 700$.

F.—Margin of pycnium in a 15-day-old infection on Wealthy. Dead epidermal cell (*a*); evacuated haustoria (*b* and *c*) occupying broken-down epidermal cells; bases of pycnosporophores (*d* and *e*) densely filled with protoplasm; intercellular hyphae (*g*) nearly empty; haustorium with dead tip (*f*) lying in a degenerated host-cell protoplast. $\times 700$.

G.—Portion of an 18-day-old lesion in a Wealthy leaf of mature age at the time of inoculation. Haustoria (*a* and *c*) partly empty and dead; hyphae (*d* and *e*) vacuolate but still alive; hypha (*b*) degenerating. $\times 1,070$.

H.—Tissue from the center of a 15-day-old infection on Yellow Transparent. Vacuolate hypha (*a*), normal hypha and haustorium (*b* and *c*), empty haustorium (*e*), injured haustorium occupying degenerating host cell protoplast at *d*. $\times 700$.

I.—Pycnial initiation in 15-day-old infection on Yellow Transparent. Collapsed epidermal cell (*a*), empty haustorium in dead host cell (*f*), vacuolate hyphae (*c*, *d*, and *e*), pycnial initials (*b*). $\times 700$.

J.—Margin of 15-day-old infection on Fameuse. Dead haustoria occupying disintegrated host cells (*a*, *b*, *d*, and *f*), with invagination of cytoplasm at *a*; dead hypha (*e*); collapsed host cell (*c*). $\times 700$.

K.—Portion of spongy parenchyma tissue beneath a pycnium from a 15-day-old infection on Wealthy. A uninucleate haustorium (*a*), thin film of host-cell cytoplasm (*c*), hypertrophied host nucleus (*b*). $\times 700$.

L.—A portion of palisade tissue beneath a pycnium in a 15-day-old infection on Wealthy. Dead haustorium (*a*), haustoria (*c*, *f*, and *h*) showing partial collapse at the tips, host nucleus (*e*) disintegrating, intercellular hyphae (*b* and *g*) evacuated. $\times 700$.

M.—Tissue from marginal zone of a 15-day-old infection on Yellow Transparent. Vacuolate hypha (*d*), normal haustoria (*a*, *b*, and *c*) occupying living cells, degenerating host cell (*e*). $\times 700$.

The palisade cells in the vicinity of the pycnia were crushed and their protoplasts were being broken down. A portion of palisade tissue beneath a mature pycnium is shown in figure 4, *L*. The host cells are slightly hypertrophied and are disorganized. Their contents appear mostly as aggregates of small globules which were not positive to any of the stains used. This indicates coagulation of the protoplasm. A host nucleus in the process of disintegration is shown at *e*. Haustoria are also in progressive stages of collapse. The one at *a* is dead. Those at *c*, *f*, and *h* have been killed back from the tip and their basal portions, aside from the presence of large nuclei, are empty. The intercellular spaces are nearly filled with empty hyphae (*b*).

The upper epidermal cells also show degeneration due to the now aggressive attack of the fungus. In figure 4, *F*, a group of these cells bordering a pycnium show prominent haustoria at *b* and *c* partly surrounded by disintegrated host-cell protoplast. The only epidermal cells to collapse completely, however, are those near the ostioles of the pycnia (*a*). The pycnosporophores (*d* and *e*) are densely filled with protoplasm and the bordering hyphae are nearly empty.

In the outer zone of infection, congeniality between the host and parasite continued to exist although marginal extension of the mycelium had practically ceased. The runners, typical of younger infections, were absent. A group of cells near the margin of a 15-day-old lesion are shown in figure 4, *E*. Haustoria at *b*, *c*, and *d* are uninucleate and contain dense cytoplasm. A vacuolate, intercellular hypha is shown at *a*. The host cells are little affected. Distinct plastids still remain.

YELLOW TRANSPARENT

Yellow Transparent was intermediate in its reaction to rust infection. The fungus became established and was able to maintain congenial relations with the host long enough to continue its gradual spread. Finally, development ceased altogether and the whole lesion collapsed before pycnia matured.

A 2-day-old infection is shown in figure 2, *II*. An arrow points to the place of entry. The primary hypha is swollen and contains one nucleus (*c*) which is dead, and a mass of dead cytoplasm at the proximal end. Otherwise, it is almost empty and apparently has ceased activity. The host nucleus at *b* is degenerating. It seems probable that the primary hypha succumbed first owing to its inability to endure the unfavorable medium surrounding it, and that the partial collapse of the host nucleus is a result of the diffusion of deleterious or toxic materials from the dead fungous protoplasm. Intercellular penetration of the palisade occurred at *a* and the secondary hypha has sent out two haustoria, which have fully expanded.

A 4-day-old infection (fig. 3, *E*) shows decided advancement. An arrow indicates the point of entry. The primary hypha (*a*) has partly collapsed and is empty. Palisade penetration occurred and the mycelium ramified the local palisade region sending typically normal haustoria into many of the cells. The relation here is congenial with very little depletion of the host cells. The mycelium has developed feebly in the spongy parenchyma. The long runners, common in infections in Wealthy leaves, are absent and the whole lesion is not more than 100 μ in diameter. A dead hypha is shown at *d* and one

in a partial state of collapse at *c*. An haustorium (*b*) is dead but its host cell shows no deleterious effects. Contact has been made with the lower epidermis at *e*. None of the host cells showed any degeneration or drastic reaction to infection, even though some of the hyphae were dead and collapsed.

Marginal spread of the fungus continued slowly until about the ninth day, when slight hypertrophy and degeneration of the host cells in the center of the lesion began. Rapid break-down of nearly all of the parasitized host-cell protoplasts followed. Only a narrow zone at the margin of the lesion showed a compatible relationship between the parasite and the host. A photomicrograph of a 15-day-old infection (pl. 1, *B*) shows that nearly all of the host-cell protoplasts in the upper-epidermis, lower-epidermis, and mesophyll tissues are degenerating or have already disintegrated.

The collapse of the host cells mentioned above followed closely upon the gradually increasing injury to the fungus. A section of a 7-day-old infection (fig. 3, *B*) shows that the palisade cells near the center of the lesion are still apparently unharmed except for a depletion of cytoplasmic substances and plastids. The host-cell nuclei still retain their normal structure and staining reaction. On the other hand, haustoria at *d* and *e* are dead. Their contents are coarsely granular and heavily stained with safranin. The haustorium at *c* has retained its normal healthy appearance.

The details of the disorganization which followed are represented in three illustrations taken from different regions of 15-day-old infections. The palisade and upper epidermal tissues in the central portion appear in figure 4, *I*. Large, densely filled hyphae (*b*) are accumulating in the subepidermal region preparatory to pycnial formation. The intercellular hyphae at *c*, *d*, and *e* are becoming evacuated, as well as the haustoria occupying collapsed palisade cells. None of the host cells is now alive and it is of special interest to note that the hyphae lying adjacent to them appear to be unharmed. A group of cells from the spongy parenchyma region (fig. 4, *H*) show widespread disintegration of host cells. Haustoria (*c* and *d*) are nearly empty and are embedded in the broken-down host-cell protoplasts. The hypha at *b* and the haustorium at *e*, however, still retain a healthy appearance. Tissue from the marginal zone is shown in figure 4, *M*. The haustoria (*a*, *b*, and *c*) are fully expanded and the invaded cells appear to be only slightly affected. The adjoining cell (*e*), however, shows hypertrophy and degeneration.

FAMEUSE

The leaves of Fameuse were resistant to rust infection. The lesions were usually confined to small regions in the upper epidermis, palisade, and spongy parenchyma tissues. Very seldom did the hyphae reach the lower epidermis. The end result was death and collapse of the host cells attacked and of the parasite.

A 2-day-old infection is shown in figure 2, *J*. The primary hypha at *b* is partly collapsed and the contents are dead. The host nucleus (*c*) shows a change in staining reaction. The chromatin material was positive to safranin rather than to the gentian-violet. This reaction is in line with that which occurred in the case of Yellow Transparent, although in Fameuse the death of the primary hypha seemed to be

more rapid. Intercellular penetration occurred at *a* and the secondary hypha has grown downward between two palisade cells but, as yet, has not produced a haustorium. The surrounding palisade and epidermal cells are unchanged.

Further contact with the host cells probably occurred soon after the second day. A 4-day-old infection (fig. 3, *F*) shows advancement of the hyphae into the second layer of palisade and spongy parenchyma tissues. Haustoria have entered several of the cells. The intercellular branch (*a* and *e*) has collapsed and most of the stainable contents now appear in the advancing hyphal tips at *d* and *g*. The palisade cell at *c*, probably the first in this region to be invaded, is dead. Other host cells are not seriously affected, although many of the haustoria are dead as shown at *f*. The primary hypha (*b*) is in a late stage of disintegration.

After the eighth day, development of the fungus ceased and the host cells and the mycelium degenerated rapidly. Analysis of the data showed that, although the fungus made only feeble growth during its existence, the actual death of the haustoria preceded serious injury to the host cells. A photomicrograph of a 15-day-old infection (pl. 1, *D*) shows that death and collapse of the tissues is nearly complete. No living hyphae or haustoria could be found. A group of cells from an 8-day-old infection (fig. 3, *A*) shows in detail the nature of the disintegration. At the margin of a 15-day-old lesion (fig. 4, *J*) dead haustoria occupy the broken-down protoplasts of their host cells at *a*, *b*, *d*, and *f* with invagination of the dead cytoplasm at *a*. In most cases the haustoria still keep their identity.

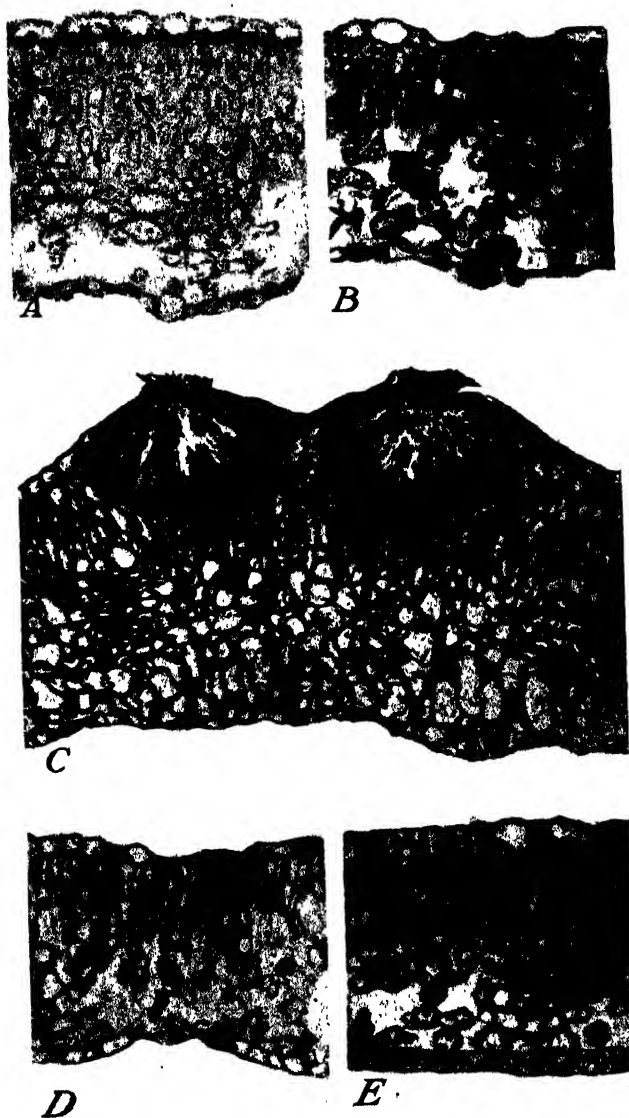
BALDWIN

The leaves of Baldwin were very resistant. The development of the fungus was restricted to the epidermal cell of entry and the adjacent palisade cells. Within 2 days after infection, the fungus died and shortly thereafter the adjoining host cells were violently affected.

A 2-day-old infection is shown in figure 2, *K*. An arrow indicates the point of entry. The primary hypha at *d* is dead. Intracellular penetration occurred and the secondary hypha (*e*) is also dead. The host nuclei at *a* and *c* within the parasitized cells are dead, and their state of collapse indicates that death followed soon after the fungus had succumbed. The host nucleus at *b* in the adjoining epidermal cell has enlarged.

A 4-day-old infection (fig. 3, *C*) shows the drastic effects upon the host cells in the lesion. The epidermal cell of entry has collapsed. It contains the dead remains of the primary hypha and of the dead host-cell protoplast. The intercellular secondary hypha is dead. A small haustorium at *a* died just as it entered a palisade cell. This cell is still alive and has just completed a division. Two palisade cells *b* are dead and a cell at *c* directly beneath these is disintegrating. All of the adjacent palisade cells contain faintly stained, vacuolated cytoplasm in contrast to the dense cell contents of the normal cells surrounding them. The plastids have disappeared and the nuclei have enlarged slightly. Here again the death of the fungus apparently preceded the collapse of the host cells and the reaction was more severe than in the case of Yellow Transparent or Fameuse.

A photomicrograph of a 3-day old infection (pl. 1, *E*) shows clearly the sharp differentiation between the dead fungus and the surrounding healthy host cells. The primary hypha and the intercellular hyphae



A, Nine-day-old infection on Wealthy, showing haustoria in many of the host cells, $\times 300$. B, Fifteen-day-old lesion on Yellow Transparent, showing general collapse of host tissues in infected area, $\times 300$. C, Fifteen-day-old infection on Wealthy, showing 2 pycnia and host reactions in their vicinity, $\times 150$. D, Fifteen-day-old infection on Fameuse, showing collapse of palisade and upper epidermal regions in the infection, $\times 150$. E, Three-day-old lesion on Baldwin, showing the dead fungus in contrast to the surrounding healthy host tissues, $\times 150$.

in the underlying palisade appear as homogenous black strands in the photograph, indicating their total collapse. The epidermal cell of entry is degenerating and a palisade cell directly beneath, which was parasitized, is dead. Adjoining palisade and upper epidermal cells appear to be unaffected. These host tissues are clearly neither hypersensitive nor hypersusceptible. Starvation of the fungus also seems to be out of the question because the hyphae did not appear to be impoverished or depleted at the time of their collapse.

FURTHER OBSERVATIONS ON WEALTHY

DORSAL-SURFACE INFECTION ON YOUNG LEAVES

Successful infection of the lower surfaces of young Wealthy leaves was infrequent. Early stages in the development of such lesions were not encountered in the cytological preparations studied. About 12 days after inoculation, however, a few scattered rust spots became visible. Cytological study of these infections showed the character of host-parasite relations to be typical for the normal development in Wealthy leaves.

In all cases, penetration of the epidermal cells occurred freely and the primary hyphae expanded in the usual manner (fig. 4, *D*). The secondary branches (*b* and *c*) penetrated the large intercellular spaces of the spongy parenchyma and, therefore, failed to establish contact with the mesophyll cells of the leaf. Cessation of growth and death quickly followed. The case here described was typical of all of the younger infections, but the occupation of a single epidermal cell by more than one primary hypha was uncommon. None of the secondary hyphae were observed to emerge into adjoining epidermal cells.

INFECTION ON LEAVES PASSING INTO MATURITY

The types of reactions shown by Wealthy leaves which had just passed the period of expansion into full maturity are of special interest. Penetration occurred through the unwounded ventral epidermis and in 3 weeks the mycelium had spread approximately 225μ from the point of entry. Hypha ramified the mesophyll tissues to a considerable extent and nearly every host cell in the lesion was invaded by a haustorium. The host cells harboring haustoria became vacuolate and their cytoplasmic substances disappeared almost completely. Pycnia were not initiated. The group of cells from an 18-day-old infection (fig. 4, *G*) show almost complete cessation of fungal activity. Haustoria at *a* and *c* are functioning no longer and the hyphae at *d* and *e*, aside from their large nuclei, are nearly empty. The hypertrophy of host cells and collapse of their protoplasts common in older infections in younger leaves is not found here.

INFECTIONS ON OLD LEAVES

Penetration into the epidermal cells did not occur on the leaves of Wealthy which were approximately 3 weeks past the attainment of full size and maturity. This was true of both dorsal- and ventral-surface inoculations. The sporidia germinated in the usual manner with the production of short germ tubes, appressoria, and infection hyphae. An examination of several hundred cases showed that none of the infection hyphae was able to complete the penetration process. Many were found, however, where these minute pegs clearly penetrated the cuticle but failed to pass the epidermal cell walls. The

staining reactions in these preparations produced clear differentiation. The infection hyphae were dark violet in contrast to the transparent, light green of the cuticle and the dark, dull green of the epidermal cell walls. In figure 4, A, the infection hypha appears to be flattened against the cuticle. The application of pressure is quite apparent because the cuticle is slightly indented and the appressorium is raised and moved to one side. In B, the infection hypha has penetrated the cuticle and indented the epidermal cell wall. Another case C shows, in addition, the partial dislodgment of the appressorium. The type of resistance shown by these leaves seems to be of a purely physical nature.

DISCUSSION

The apple rust fungus is an obligate parasite and goes through its cycle of development only upon a living, susceptible host. When an apple leaf is exposed to infection under favorable external conditions, the paramount factors requisite to a successful infection, therefore, seem to fall naturally into three classes, as follows: (1) The ability of the sporidial germ tube to penetrate into the epidermal cell of the leaf, (2) the presence of a suitable substrate for the growth of the parasite in the cell protoplasts, and (3) the ability of the host cells to remain alive and function in a manner agreeable to the fungus. If this is true, any shortcomings in the production of a successful infection may be attributed to the absence of one or more of these requisites.

It has been shown that the apple rust fungus was able to penetrate the leaves of resistant varieties. No evidence was obtained which indicated that the parasite was excluded, even from the leaves of Baldwin, where no macroscopic signs of infection were detected. Gibson (18) observed that uredinial germ tubes entered the stomata of plants widely separated taxonomically, and concluded that penetration is not a reliable index of infecting capacity. This may have no application here, however, because the sporidial germ tubes of *Gymnosporangium juniperi-virginianae* penetrate directly. Melander and Craigie (26) found, in some cases, a correlation between resistance of the epidermis to puncture and rust resistance in the leaves of *Berberis*, the alternate host of *Puccinia graminis*. They found no evidence that the sporidial germ tubes entered an immune host like *B. thumbergii*, and concluded that morphological characters may account for resistance in some species. Although the observations made upon apple rust lend no support to the existence of varietal resistance due to morphological features, it is not impossible that such cases may be found in other varieties.

The immature leaves of Wealthy are susceptible to *G. juniperi-virginianae*. As long as the host cells remain alive they present a favorable medium for the development of the parasite. Dead haustoria are never found associated with living cells of this variety.

Varietal resistance appears to be due to some factor or factors in the host-cell protoplasts injurious to the fungus. This antagonism is severe in Baldwin, slightly less pronounced in Fameuse, mild in Yellow Transparent, and totally absent in Wealthy. The exact nature of the factor or factors involved still awaits solution and may be sought in an analysis of the chemical composition and physicochemical structure of the living host cells.

In lieu of an actual solution of the problem, various theories have been suggested. Many of these are based entirely upon microscopical evidence, but, recently, other methods of study have been employed. Ward (34) proposed that immunity was due to antagonistic reactions between host and parasite of the nature of the formation of toxins and antitoxins. Marryat (25), Gibson (18), Stakman (33), Allen (1), and others are generally in agreement with Ward. Leach (22) suggested a hypothesis based on a possible specific food requirement on the part of the fungus and the corresponding lack of this food substance within the host. Edgecombe (15) performed serological studies of wheats, susceptible and resistant to *Puccinia rubigo-vera tritici*. Purified globulin extracts of the grain were used as immunizing agents and the resulting antisera were given the precipitin test using the original extracts as test antigens. The results showed a relationship between precipitin reaction and rust resistance. Since the globulins used were believed to have been a part of the cytoplasm, it was reasoned that the genetic factors responsible for resistance were in some way associated with the factors producing the specific globulins. Leemann (24) proposed the theory that the holistic quality of the cell may play an important role in active plant immunity. It was based upon the assumption that the protoplasm of an immune host may not contain any specific substance deleterious to the invading organism, but that the living protoplasm as a whole, built up into a complex system, may present an unfavorable substrate. The phenol hypothesis of Newton and Anderson (28) suggests that rust resistance in wheat may be caused by the liberation of toxic phenolic compounds in the host cell upon the entrance of the fungus. This mechanism may explain different types of rust reactions when one considers the possible conditions following the action of fungal enzymes upon the phenolic compounds in the host-cell protoplasts.

Whether the antagonism against the apple rust fungus is already present in the make-up of the host-cell protoplasts or is induced by the parasite and presents a counterreaction against the intruder is open to question. Cytological evidence cannot settle this point. However, the substance or substances antagonistic to the fungus seem to be specific because many cases were found where the haustorium occupying the host cell had died and the cell itself showed no visible change. The reverse situation was found only in the older infections upon Wealthy and Yellow Transparent where the host apparently succumbed to the vigorous and prolonged attack by the parasite.

Regarding the interaction of host and parasite, many workers have observed peculiarities in the sequence of events. Ward (34), studying infections by *Puccinia dispersa* in resistant bromes, found that the germ tubes entered the stomata in a normal manner but either killed a few cells and then disintegrated or grew very slowly and failed to produce pustules. Marryat (25), working with *P. glumarum* upon the resistant American Club wheat, also noted irregularity. Stakman (33) observed injury of host cells to be a common phenomenon among plants resistant and immune to *P. graminis* and that the degree of hypersensitiveness varied in direct proportion to the degree of resistance. Regarding infection of *P. graminis tritici* upon the resistant Kanred wheat, Allen (1, p. 148) says, " * * * in one case the host cell is severely damaged at a time when the fungus is not visibly harmed, and in another the host cell is still normal in appearance

while the fungus has succumbed." According to Evans (17) no harmful effects to the host cells followed the invasion or establishment of the onion smut fungus, *Urocystis cepulae*, in either susceptible or nearly immune onion seedlings. In fact, mycelium which was badly degenerated or already dead was found in host cells which were still apparently healthy.

During active production of the pycnial exudate by the apple rust fungus, the mycelium seemed drained of its cytoplasm. Evans (16) and Allen (2), working with certain of the cereal rusts, noted a drainage of protoplasm from the central mycelium to the peripheral hyphae during fruiting. This does not seem to apply here because an accumulation of protoplasm in the peripheral hyphae is not found. A likely interpretation is that the contents of the hyphae are transported in some manner to the pycnia to support the prolific fruiting of the fungus. This is in agreement with the recent work of Liu⁴ who made similar observations during his investigations of sexuality in *Gymnosporangium juniperi-virginianae*. The mechanism of this transfer is not understood, although it probably depends upon the presence of pores or other openings in the septa and a consistency of cytoplasm which would expedite movement. Allen (2) recognized this problem and suggested the possibility of some autolytic or digestive process reducing the hyphal contents to simpler soluble forms that would be more readily transportable. For a long time it has been known that protoplasmic streaming occurs in fungi, especially in the mucors and other groups having coenocytic hyphae. Buller (13) gives an excellent review of the literature on this subject. His own research showed that, in the higher fungi, pores were present in the septa through which protoplasm passed freely. Pores were not observed in the septa of the apple rust fungus. If, however, what Buller observed is applicable here, the passage of fungous protoplasm to a point where it is needed most may be explained.

It is also of interest that the marginal spread of the lesion almost ceased when pycnial formation began. Here again, it is believed that the fruiting activities of the fungus drew so heavily upon the food materials of the hyphae that energy for continued vegetative growth was not available.

Inoculations upon the dorsal surfaces of young Wealthy leaves usually failed. The secondary hyphae, emerging from the epidermal cells into the large intercellular spaces of the spongy parenchyma region, became shriveled and finally collapsed before they could establish contact with mesophyll cells. Death of the fungus by starvation seems to offer the most plausible explanation for this type of infection failure. In addition, the protoplasts of the lower epidermal cells may present a less suitable medium for the young parasite and the secondary hyphae possibly find the changing gas tensions of the intercellular spaces unfavorable during early development. The parasite certainly encounters a different environment in the lower epidermis as compared to the upper where 2 or 3 compact palisade layers without apparent intercellular spaces are present. It is, therefore, not surprising that the parasite behaves differently following lower-surface penetration.

⁴LIU, J. C. INVESTIGATIONS ON THE SEXUAL BEHAVIOR OF THE APPLE RUST FUNGUS. Unpublished doctoral dissertation. Univ. Wis. 1933.

The nature of resistance of mature Wealthy leaves is of special interest in view of the present lack of cytological evidence concerning this phenomenon.

Giddings (19) reported that York Imperial apple leaves were usually susceptible to rust for from 15 to 25 days after they unrolled from the buds, the length of the period being closely associated with growth conditions, and believed (19, p. 33)

* * * this acquired immunity in York Imperial leaves is due primarily to a change in materials available as food for the early nutrition of the fungus; and secondarily to certain changes in the thickness and composition of cell walls, leaf coverings, etc., which might be classed as physical.

Regarding this point, Reed and Crabill (30) also advanced the probability that the inhibiting factor was the increasing thickness of the cuticle and cell walls.

Giddings and Berg (20) found that mature leaves occasionally became infected through insect punctures and other wounds, but that infections of this kind developed very small lesions and aecia were never noted from them. This may lead to the belief that penetration was normally prevented but occurred when an avenue of entry was provided, and that, once inside the tissues, the fungus was unable to develop very far, due to some unfavorable situation in the mature leaf mesophyll.

The inoculation experiments of Miller (27) showed that sound, mature leaves of the susceptible varieties, Rome Beauty, Wealthy, and Jonathan, were resistant to apple rust infection but when the leaves were torn, prior to inoculation, lesions producing pycnia and aecia occurred along the edges of the wound. The mature leaves of the resistant varieties, Winesap and Delicious, showed no infection even when wounded. These data led to the interpretation that the resistance shown by mature leaves of susceptible varieties probably possessed a morphological basis.

In the present work, Wealthy leaves, which had reached full size at the time of inoculation, showed no visible infection. Microscopic examination, however, showed the presence of numerous lesions. Although actual penetration was not observed, no wounds were found near the point where entry was believed to have occurred. Some factor or factors prevented the fungus from following its normal cycle of development. A fundamental antagonism apparently does not exist. The evidence on this point is clear but interpretation is difficult and anything that might be said about it would be purely speculative.

The sporidial germ tubes failed to penetrate the epidermis of old Wealthy leaves. In some cases the infection hyphae punctured the cuticle, only to be stopped by the epidermal cell wall. In many instances, the cuticle and epidermal cell wall were indented and the appressorium partly dislodged from the leaf. These observations strongly suggest a physical ability of the epidermis to withstand the pressure of the penetrating infection hyphae.

SUMMARY

Cytological studies of apple rust infections in the leaves of four apple varieties, each differing from the other in its reaction to the rust fungus, are here reported.

The sporidial germ tubes penetrated the host directly and the process was essentially the same on each variety. The fungus began its parasitic activities intracellularly by developing a conspicuous primary hypha in an epidermal cell.

In young Wealthy leaves, the fungus developed vigorously for about 10 days after infection occurred. The uninucleate mycelium was regularly intercellular, sending characteristically uninucleate haustoria into the host cells. The haustoria were usually simple, rarely branched. Impoverishment of the parasitized host cells was slight, resulting generally only in a reduction in number of plastids.

Coincident with pycnia formation, marginal spread of the fungus almost ceased and the hyphae appeared to be partly depleted of their cytoplasmic contents. These phenomena are attributed to the fruiting activities of the fungus. It is thought that the food reserves in the hyphae, transported centripetally in some way to support the rapid exudation of the pycnia, were unavailable for further vegetative growth.

In the vicinity of the pycnia, the spongy parenchyma cells became hypertrophied and the protoplasts of the palisade and upper epidermal cells degenerated. The hypertrophy resulted in the obliteration of the large intercellular spaces in the spongy parenchyma region and the collapse of a portion of the lower epidermis. Haustoria occupying the broken-down cells were in various stages of collapse due to their unfavorable surrounding. As long as the host cells remained alive, however, they furnished a favorable substrate for fungal development.

In the leaves of the other varieties used, resistance was due to a distinct antagonism of the host-cell protoplasts to the invading parasite. The reaction was mild in Yellow Transparent, severe in Fameuse, and extremely severe in Baldwin. The failure of the fungus to establish itself could not be attributed to hypersensitiveness of the host cells, resulting in starvation of the obligate parasite. Death and collapse of the fungus preceded injury to the host cells.

Dorsal-surface infections upon young Wealthy leaves generally failed. Penetration occurred in the usual manner but the secondary hyphae, emerging from the epidermal cells of entry into the large intercellular spaces of the spongy parenchyma tissue, shriveled and collapsed before they could establish contact with mesophyll cells. This infection failure is attributed largely to starvation of the parasite.

As Wealthy leaves reached mature size, they became resistant. No macroscopic lesions appeared. The fungus, however, was able to penetrate and make considerable vegetative growth. Pycnia were not initiated. Aside from depletion of the invaded cells, the host showed little deleterious effects. Some situation, apparently, in the host, did not allow the fungus to follow its normal cycle of development.

The complete resistance shown by old Wealthy leaves is attributed to physical properties of the epidermis which successfully prevented the infection hyphae from gaining entrance into the host.

LITERATURE CITED

(1) ALLEN, R. F.

1923. A CYTOLOGICAL STUDY OF INFECTION OF BAART AND KANRED WHEATS BY PUCCINIA GRAMINIS TRITICI. *Jour. Agr. Research* 23: 131-152, illus.

- (2) ALLEN, R. F.
1924. CYTOLOGICAL STUDIES OF INFECTION OF BAART, KANRED, AND MINDUM WHEATS BY PUCCINIA GRAMINIS TRITICI FORMS III AND XIX. Jour. Agr. Research (1923) 26: 571-604, illus.
- (3) ———
1926. CYTOLOGICAL STUDIES OF FORMS 9, 21 AND 27 OF PUCCINIA GRAMINIS TRITICI ON KHAPLI EMMER. Jour. Agr. Research 32: 701-725, illus.
- (4) ———
1926. A CYTOLOGICAL STUDY OF PUCCINIA TRICINA PHYSIOLOGIC FORM 11 ON LITTLE CLUB WHEAT. Jour. Agr. Research 33: 201-222, illus.
- (5) ———
1927. A CYTOLOGICAL STUDY OF ORANGE LEAF RUST, PUCCINIA TRITICINA PHYSIOLOGIC FORM 11, ON MALAKOFF WHEAT. Jour. Agr. Research 34: 697-714, illus.
- (6) ———
1928. A CYTOLOGICAL STUDY OF PUCCINIA GLUMARUM ON BROMUS MARGINATUS AND TRITICUM VULGARE. Jour. Agr. Research 36: 487-513, illus.
- (7) ———
1930. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA GRAMINIS. Jour. Agr. Research 40: 585-614, illus.
- (8) ———
1932. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA TRITICINA. Jour. Agr. Research 44: 733-754, illus.
- (9) ———
1932. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA CORONATA. Jour. Agr. Research 45: 513-541, illus.
- (10) ———
1934. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN FLAX RUST. Jour. Agr. Research 49: 765-791, illus.
- (11) BIFFEN, R. H.
1907-12. STUDIES IN THE INHERITANCE OF DISEASE-RESISTANCE. I-II. Jour. Agr. Sci. [England] 2: [109]-128, 1907; 4: [421]-429, 1912.
- (12) BLISS, D. E.
1933. THE PATHOGENICITY AND SEASONAL DEVELOPMENT OF GYMNOSPORANGIUM IN IOWA. Iowa Agr. Expt. Sta. Research Bull., 166 pp. [339]-392, illus.
- (13) BULLER, A. H. R.
1933. RESEARCHES ON FUNGI. v. 5, illus. London.
- (14) COONS, G. H.
1912. SOME INVESTIGATIONS OF THE CEDAR RUST FUNGUS, GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE. Nebr. Agr. Expt. Sta. Ann. Rept. 25: [215]-245, illus.
- (15) EDGECOMBE, A. E.
1931. IMMUNOLOGICAL RELATIONSHIPS OF WHEAT RESISTANT AND SUSCEPTIBLE TO PUCCINIA RUBIGO-VERA TRITICINA. Bot. Gaz. 91: 1-21.
- (16) EVANS, I. B. POLE
1907. THE CEREAL RUSTS. I. THE DEVELOPMENT OF THEIR UREDO MYCELIA. Ann. Bot. [London] 21: [441]-466, illus.
- (17) EVANS, R. I.
1933. CYTOLOGICAL STUDIES ON THE PARASITIC RELATIONSHIP OF UROCYSTIS CEPULAE TO THE ONION. Amer. Jour. Bot. 20: 255-268, illus.
- (18) GIBSON, C. M.
1904. NOTES ON INFECTION EXPERIMENTS WITH VARIOUS UREDINEAE. New Phytol. 3: 184-191, illus.
- (19) GIDDINGS, N. J.
1918. INFECTION AND IMMUNITY IN APPLE RUST. W. Va. Agr. Expt. Sta. Bull. 170, 71 pp., illus.
- (20) ——— and BERG, A.
1915. APPLE RUST. W. Va. Agr. Expt. Sta. Bull. 154, 73 pp., illus.
- (21) KEITT, G. W., and JONES, L. K.
1926. STUDIES OF THE EPIDEMIOLOGY AND CONTROL OF APPLE SCAB. Wis. Agr. Expt. Sta. Research Bull. 73, 104 pp., illus.

- (22) LEACH, J. G.
1919. THE PARASITISM OF PUCCINIA GRAMINIS TRITICI ERIKSS. AND HENN. AND PUCCINIA GRAMINIS TRITICI-COMPACTI STAK. AND PIEM. *Phytopathology* 9: [59]-88, illus.
- (23) ———
1923. THE PARASITISM OF COLLETOTRICHUM LINDEMUTHIANUM. *Minn. Agr. Expt. Sta. Tech. Bull.* 14, 41 pp., illus.
- (24) LEEEMANN, A. C.
1932. THE PROBLEM OF ACTIVE PLANT IMMUNITY. *Zentbl. Bakt. [etc.]* (II) 85: 360-376.
- (25) MARRYAT, D. C. E.
1907. NOTES ON THE INFECTION AND HISTOLOGY OF TWO WHEATS IMMUNE TO ATTACKS OF PUCCINIA GLUMARUM, YELLOW RUST. *Jour. Agr. Sci. [England]* 2: [129]-138, illus.
- (26) MELANDER, L. W., and CRAIGIE, J. H.
1927. NATURE OF RESISTANCE OF BERBERIS SPP. TO PUCCINIA GRAMINIS. *Phytopathology* 17: 95-114, illus.
- (27) MILLER, P. R.
1932. PATHOGENICITY OF THREE RED-CEDAR RUSTS THAT OCCUR ON APPLE. *Phytopathology* 22: 723-740, illus.
- (28) NEWTON, R., and ANDERSON, J. A.
1929. STUDIES ON THE NATURE OF RUST RESISTANCE IN WHEAT. IV. PHENOLIC COMPOUNDS OF THE WHEAT PLANT. *Canad. Jour. Research* 1: 86-99, illus.
- (29) PEACE, L. M.
1910. NOTES UPON THE CLEARING AND STAINING OF LEAVES AND STEMS. *Plant World* 13: 93-96.
- (30) REED, H. S., and CRABILL, C. H.
1915. THE CEDAR RUST DISEASE OF APPLES CAUSED BY GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE SCHW. *Va. Agr. Expt. Sta. Tech. Bull.* 9, 106 pp., illus.
- (31) RICE, M. A.
1927. THE HAUSTORIA OF CERTAIN RUSTS AND THE RELATION BETWEEN HOST AND PATHOGENE. *Bull. Torrey Bot. Club* 54: 63-153, illus.
- (32) RUTTLE, M. L., and FRASER, W. P.
1927. A CYTOLOGICAL STUDY OF PUCCINIA CORONATA CDA. ON BANNER AND COWRA-35 OATS. *Calif. Univ. Pubs., Bot.* 14: 21-72, illus.
- (33) STAKMAN, E. C.
1915. RELATION BETWEEN PUCCINIA GRAMINIS AND PLANTS HIGHLY RESISTANT TO ITS ATTACK. *Jour. Agr. Research* 4: 193-200, illus.
- (34) WARD, H. M.
1902. ON THE RELATIONS BETWEEN HOST AND PARASITE IN THE BROMES AND THEIR BROWN RUST, PUCCINIA DISPERSA (ERIKSS.). *Ann. Bot. [London]* 16: [233]-315, illus.
- (35) ———
1903. FURTHER OBSERVATIONS ON THE BROWN RUST OF THE BROMES, PUCCINIA DISPERSA (ERIKSS.), AND ITS ADAPTIVE PARASITISM. *Ann. Mycol.* 1: [132]-151.
- (36) ———
1905. RECENT RESEARCHES ON THE PARASITISM OF FUNGI. *Ann. Bot. [London]* 19: 1-54.
- (37) WATERHOUSE, W. L.
1921. STUDIES IN THE PHYSIOLOGY OF PARASITISM. VII. INFECTION OF BERBERIS VULGARIS BY SPOIDIA OF PUCCINIA GRAMINIS. *Ann. Bot. [London]* 35: [557]-564, illus.
- (38) WEIMER, J. L.
1917. THREE CEDAR RUST FUNGI; THEIR LIFE HISTORIES, AND THE DISEASE THEY PRODUCE. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 390, pp. 507-549, illus.
- (39) ZIMMERMANN, A.
1925. SAMMELREFERATE ÜBER DIE BEZIEHUNGEN ZWISCHEN PARASIT UND WIRTS-PFLANZE. II. DIE UREDINEEN. *Zentbl. Bakt. [etc.]* (II) 65: 311-418, illus.
- (40) ZIRKLE, C.
1930. THE USE OF N-BUTYL ALCOHOL IN DEHYDRATING WOODY TISSUE FOR PARAFFIN EMBEDDING. *Science (n. s.)* 71: 103-104.

INFLUENCE OF THE CARBOHYDRATE-NITROGEN RELATION ON NODULE PRODUCTION BY RED CLOVER¹

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INTRODUCTION

There are many factors which may influence the production of nodules upon the roots of leguminous plants. Some of these are physical in character, such as light intensity, soil temperature, texture, and moisture; others are chemical in nature, such as soil acidity, inorganic salts, and simple or complex organic nitrogenous compounds. Of fundamental interest is one of a chemical character relating to the presence of certain inorganic salts in the substrate, viz, the quantity and kind of combined nitrogen.

The presence of nitrates and ammonium salts was known to reduce or completely suppress the number of nodules on inoculated legumes long before the experiments of Hellriegel and Wilfarth revealed the relationship between the centers of nitrogen fixation (the nodules) and their causal agents (the bacteria). The problem has attracted such wide interest that even to the present day researches have been conducted in the hope of determining the means by which the nodules are suppressed. A vast accumulation of data has resulted, but until recently attempts at interpretation have met with little success.

LITERATURE

During the 70 years that the inhibitory effect of inorganic nitrogen on legume nodulation has been recognized, many theories have been proposed to explain it, but the majority of these are supported by little or no experimental evidence. Briefly, the earlier theories were concerned with the toxic effect of nitrate nitrogen on the root-nodule bacteria, the production of a toxic principle in the plant, and immunity of the plant.

Mazé (11)² suggested that the combination of inorganic nitrogen with carbohydrate in the plant to form organic nitrogenous compounds effectively reduces the level of carbohydrate. As a result, excretion of carbohydrate from the roots is decreased and its chemotactic action on the bacteria reduced. Previous papers from this laboratory (7, 9, 12) have presented data which are readily explained by a modification of Mazé's theory; the modified hypothesis stresses the necessity of adequate carbohydrate supply in the interior of the plant rather than a hypothetical chemotactic effect. A paper of Fred and Wilson (6) summarizes the experimental results and the hypothesis offered in explanation.

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² Reference is made by number (italics) to Literature Cited, p. 611.

In field experiments, Rippel and Krause (14) show that the carbohydrate demands of the roots of the pea plant are much higher if nodules are present. The destruction of carbohydrate by the pea roots without nodules was similar to that of a nonleguminous plant. These observations are in complete agreement with the modified theory. Allison and Ludwig (1) reemphasize the importance of carbohydrate supply on nodule formation and suggest that the primary need of the carbohydrate is for tissue development rather than for energy purposes. They stress the increase in the top:root ratio with the addition of nitrogen, and affirm that nodule development is repressed by combined nitrogen for the same reason that root development is relatively decreased under the same treatment.

Hypotheses in explanation of this fundamental property of the fixation process in leguminous plants have been based for the most part on physiological experiments and have suffered from lack of experimental data of a biochemical nature. So far as the writer is aware, critical experiments to test the adequacy of a given hypothesis have not been made or even suggested by the various authors. Until these data are available, speculation is likely to replace conclusions based on experimental facts. This paper offers biochemical evidence bearing on the carbohydrate supply hypothesis gained from experiments of the following types:

(1) The carbohydrate supply with respect to the nitrogen was varied by regulating the $p\text{CO}_2$ ³ in the air that was supplied to red-clover plants, and the effect on the nodulation of the plants was studied.

(2) The level of carbohydrate in tops and roots of clover plants in which the carbohydrate-nitrogen relation varied was determined and correlated with invasion of the plant by bacteria and the development of the resulting nodules.

EXPERIMENTAL TECHNIQUE

CELLOPHANE CHAMBER

A suitable chamber for growth of plant cultures was devised, the details of which are shown in figure 1. The container is 20 inches high and 7 inches in diameter. It is covered with waterproof cellophane. The tin frame is sealed to a shallow porcelain pan by calking with plugging cotton and saturating the cotton with hot paraffin. A tube delivers the desired air plus CO_2 mixture to each pot after the mixture leaves the manifold. Details of the mechanics of mixing the gases for suitable atmospheres, filtering them, regulating their flow, and delivering them to the manifolds are given in a paper by Wilson and Georgi (20).

The growth of the plants in this type of container is as good as that of plants grown in open pots in the greenhouse. The chambers effectively keep out gross contamination without restricting growth or interfering with transpiration. Diffusion of CO_2 from a chamber is sufficiently slow so that concentration of this gas is higher than that of the surrounding air and can thus be readily maintained inside the container.

³ $p\text{CO}_2$ = partial pressure of CO_2 in atmosphere.

PLANT CULTURES

Plants of red clover (*Trifolium pratense*, var. Mammoth) were grown in sterilized half-gallon earthenware pots filled with a thin layer of gravel over which nitrogen-free pit sand was placed. A 25 by 200 mm glass tube in the center of the pot served as a means of watering; the surface of the sand was covered with sterile cork, which helped to prevent contamination by algae or micro-organisms.

The seeds were sterilized in Dakin's solution and germinated on sterile moist blotters in Petri dishes after the method of Hopkins, Wilson, and Fred (8). After the seeds had been allowed to germinate for 48 hours on the blotters, 10 to 12 seedlings were transferred aseptically to each pot. The seedlings were inoculated with a

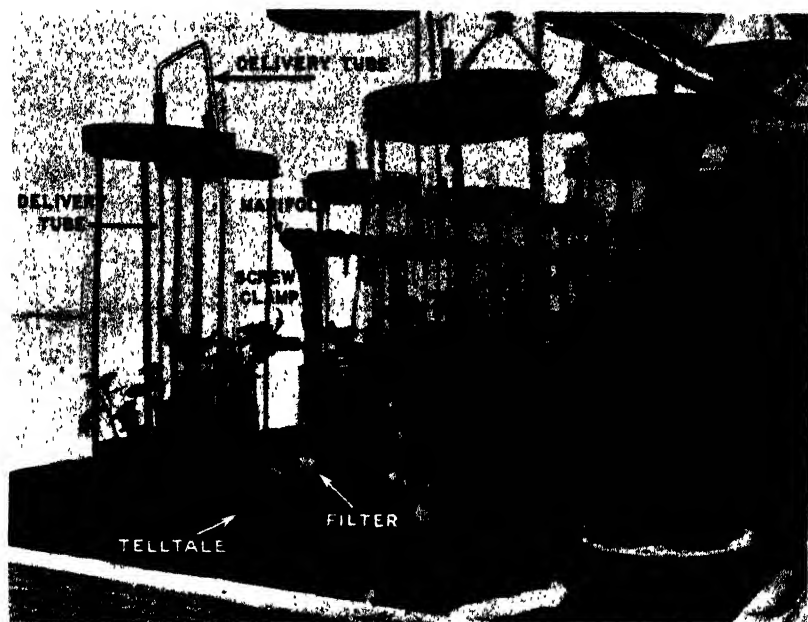


FIGURE 1.—Detail of cellophane chambers used for growing plant cultures.

water suspension of a 48-hour culture of *Rhizobium trifolii*. Strain 209, an effective one, was used. Loss of moisture from the pots was frequently checked, sterile water or nitrogen-free Crone's (3) solution being used to bring them up to weight. The nitrate nitrogen additions were made as sterile solutions in concentrations varying from 2.25 mg to 3.0 mg per cubic centimeter of solution.

Carbon-dioxide analyses of the atmospheres inside the cellophane chambers were made several times a week by methods developed in this laboratory (16, 19, 21). Atmospheres in the CO₂ series were found to vary from 0.1 to 0.25 percent. Sunlight was supplemented with artificial light during the fall, winter, and early spring months, as well as on cloudy days. The greenhouse temperature remained at about 25° C. throughout all of the experimental work.

HARVESTING AND CHEMICAL ANALYSES

The plants were washed from the pots at the time of harvest, special care being taken not to lose any of the nodules. After the nodules had been counted, the tissue was dried at 38° C. for 48 hours and the dry weight determined. The tissue was subsequently ground for analysis.

One-half of the plants used in experiments 5 to 7 were harvested 2 hours after sundown, at the close of the fifth or sixth week; the remainder, 10 days later. The roots were separated from the tops and the wet weight of each recorded. Both were then cut up into small bits, care being exercised to keep the loss of plant sap to a minimum. The samples for analysis were weighed as rapidly as possible to prevent the loss of moisture.

Total nitrogen determinations were made in experiments 1 to 4 on 0.75-g samples, as outlined for plant tissue in the Association of Official Agricultural Chemists' manual (2). In the presence of nitrate nitrogen the method recommended by Phillips et al. (13) was used. Nitrate nitrogen was determined on 1-g samples of dry tissue by the reduction method of Sessions and Shive (15).

In experiments 5 to 7, nitrate nitrogen was determined on 1-g portions of wet tissue immediately after harvest by the colorimetric method of Emmert (4). The total soluble carbohydrate fraction was analyzed according to the procedure recommended by Loomis et al. (10). Reducing sugars were determined by the micromethod of Stiles, Peterson, and Fred (17).

EFFECT OF INCREASED $p\text{CO}_2$ ON NODULE PRODUCTION BY CLOVER GROWN IN THE PRESENCE OF COMBINED NITROGEN

If the inhibiting effect of nitrate nitrogen on nodule production is correlated with the removal of the available total soluble carbohydrate by the inorganic nitrogen for protein synthesis, then the inhibition should be overcome if the sugar level in the plant sap is raised. Four experiments were made in which the $p\text{CO}_2$ in the atmosphere supplied inoculated clover plants furnished with various levels of combined nitrogen was increased in order to raise the carbohydrate content of the plant. Thus a critical test of the carbohydrate supply hypothesis could be obtained.

EXPERIMENT 1

These plants used in the first experiment were grown for 58 days in the fall of 1932. Ten milligrams of KNO_3 per eight plants per bottle were added at the start of the experiment and 10 milligrams weekly thereafter until the level of nitrate designated was reached. The first experiment was the only one not carried out in cellophane cans; instead, open 64-ounce flint glass bottles were employed, as described by Wilson and Georgi (20). Fifteen days after planting, CO_2 was started through the air plus CO_2 series.

The appearance of the tops (stems and leaves) was typical of the plants in subsequent experiments. The following details hold for all the experiments. Plants grown in a normal atmosphere were markedly smaller in height and leaf area than plants of the same NO_3 level enveloped in an atmosphere of increased $p\text{CO}_2$. The

color of the plants receiving nitrate nitrogen was a much deeper green than that of the controls (or 0-mg level), those exposed to the higher $p\text{CO}_2$ being a deeper green than those exposed to the normal $p\text{CO}_2$ of the air.

At the time of harvest the following observations were made with respect to nodule distribution. On the roots of plants of the 0-mg air series, nodules were fingerlike in shape and clustered around the crown of the taproot. In the 10-mg air series much the same picture was presented, but in the 20-mg air series nodules were small and round, indicating the "antagonistic" or inhibiting effect of the nitrate. In both the 40- and 80-mg air series, all of the nodules were small and round; at the latter level many plants possessed no nodules. In the air plus CO_2 series, a very different distribution took place. Plants at the 0-mg level possessed several very large fingerlike nodules on the crown of the taproot, and even the secondary roots contained nodules, both round and small fingerlike ones. At the 10-mg level, nodule distribution was very similar to that at the 0-mg level, except that more small, round nodules were in evidence. At the 20-mg level there were very few nodules on the taproot, the majority being relegated to the secondary root system. At both the 40- and 80-mg levels, the nodules were all on the secondary roots and small in size.

TABLE 1.—Effect of increased $p\text{CO}_2$ on nodule production by red clover in the presence of KNO_3 ,¹ in experiment 1 (58 days), Sep. 28 to Nov. 25, 1932

Treatment		Plants				Nodules					
Atmosphere ²	KNO_3	Total	Dry weight per 10—	Total nitrogen per 10 —		Total count		Per grams (dry weight)		Per 100 milli-grams of nitrogen	
	Milli-grams	Num-ber	Grams	Milli-grams	Per-cent	Number	Per-cent ⁴	Num-ber	Per-cent ⁴	Num-ber	Per-cent ⁴
Air.....	0	88	0.808	16.8	2.08	121±13.1	100	150	100	720.2	100
	10	56	.785	17.4	2.21	90±4.4	74.4	115	76.7	517.2	71.8
	20	55	.724	16.0	2.21	63±5.8	52.0	87	58.0	393.7	54.7
	40	60	.654	17.2	2.62	32±4.2	26.5	49	32.7	196.1	25.5
	80	60	.725	18.7	2.71	3±1.8	2.5	4.1	2.7	15.2	2.1
CO_2	0	83	1.476	24.7	1.68	145±8.5	100	98	100	587.0	100
	10	59	1.015	21.7	2.14	111±11.0	76.6	109	111.2	511.5	87.1
	20	77	1.033	21.3	2.06	90±8.2	62.1	87	88.8	422.9	72.0
	40	48	1.032	24.7	2.39	51±3.9	35.2	49	50.0	206.5	35.2
	80	60	.908	20.2	2.22	33±10.2	22.8	36	36.7	163.4	27.8

¹ 10.0 mg added weekly to each pot; 10.0 mg added at start.

² CO_2 atmosphere contained approximately 0.15 percent CO_2 .

³ Nitrate nitrogen-free.

⁴ Zero-mg level = 100 percent.

Table 1 gives the data pertinent to this experiment. There is marked evidence of the stimulatory effect of added CO_2 in both the total weight of nitrogen and in the average dry weight per 10 plants. The percentage of nitrogen is somewhat smaller in the air plus CO_2 series than in the air, indicating that the carbohydrate relative to nitrogen is higher in plants of this series. The total count of nodules per 10 plants is calculated in this table with the standard deviation.

A comparison of the total counts shows that plants receiving increased $p\text{CO}_2$ always possessed a greater number of nodules than the corresponding plants of the air atmosphere, i. e., plants re-

ceiving the same quantity of combined nitrogen. In all cases, the addition of NO_3 caused a reduction in the number of nodules, but the reduction was relatively less on the plants receiving additional CO_2 . This is best brought out by comparison of the relative number of nodules on the plants of each NO_3 level with the 0-mg level equal to 100 in both the air and CO_2 series. In every case the relative number of nodules in corresponding levels of NO_3 was greater in the CO_2 series. This shows conclusively that the reduction was less in the CO_2 series.

In order to correct for the differences in size of plants, calculations were made on the number of nodules per gram of dry weight and per gram of nitrogen. Once again, comparing the relative values, it is seen that the reduction in the number of nodules (because of the presence of NO_3) is relatively smaller in the CO_2 series.

Attention is called to the fact that the absolute number of nodules per unit of weight or unit of nitrogen in the absence of nitrate nitrogen in the air series is greater, indicating that the centers of fixation (nodules) are more active on the plants receiving additional CO_2 . This might arise from two factors: (1) Larger nodules, and hence a greater amount of tissue for fixation activity, or (2) the higher level of soluble carbohydrate that is probably available to the cells of the roots of the plants for tissue synthesis.

Under "total count", the stimulatory effect of the air plus CO_2 series (referred to from now on as the CO_2 series) over the air series is very marked, less so at the lower levels of nitrate. In the case of the highest level, 80 mg, the increase in the number of nodules of the CO_2 series over that of the air series is more than tenfold. The inhibitory effect of the nitrate nitrogen is evident within both the air and the CO_2 series, but it is to some extent overcome by an increase in pCO_2 furnished the plants. The same holds true for the number of nodules per gram of dry weight and the number per gram of nitrogen. The stimulatory effect of CO_2 calculated from the number of nodules per gram of nitrogen is marked, as can be seen by comparing the data shown for the two series in the last column of table 1.

EXPERIMENT 2

The plants in experiment 2 were harvested on the fifty-ninth day; they were grown during the winter months. The combined nitrogen was added as NH_4NO_3 to pots of 10 plants each. The following levels were used 0, 25, 50, and 100 mg per 10 plants. No nitrate was added to any of the plant cultures until the seventeenth day, when 25 mg was added to each of the NO_3 pots. Weekly thereafter, 25 mg was added to each of the 50- and 100-mg levels until the requisite quantity had been reached. Carbon dioxide was passed through the CO_2 series beginning with the seventeenth day of growth.

At the time of harvest the number of nodules within each series was found to decrease progressively with increasing nitrate nitrogen in all treatments; however, the number of nodules was greater in the CO_2 series. The plants in both 0-mg series had fingerlike nodules near the crown of the taproot, but those exposed to increased

$p\text{CO}_2$ possessed more and larger nodules, many of which were distributed over the secondary root system. The large fingerlike nodules found on the 0-mg CO_2 series were colored pink (anthocyanin pigments), characteristic of plant tissue exhibiting carbohydrate excess. In general, the effect of the NH_4NO_3 on nodule size and distribution was similar to that observed in experiment 1.

Figure 2 summarizes the essential data of experiment 2. At each of the nitrate levels the CO_2 series had almost twice as many nodules per 10 plants as the corresponding air series. A decrease in the total count within each series is evident with increasing nitrate nitrogen. Data not included in the graph showed that there was a marked increase in total nitrogen and dry weight in the CO_2 series, again almost twice that of the corresponding levels in the air series.

Figure 3 shows the characteristic distribution of nodules and the types of root systems of plants exposed and of plants not exposed to

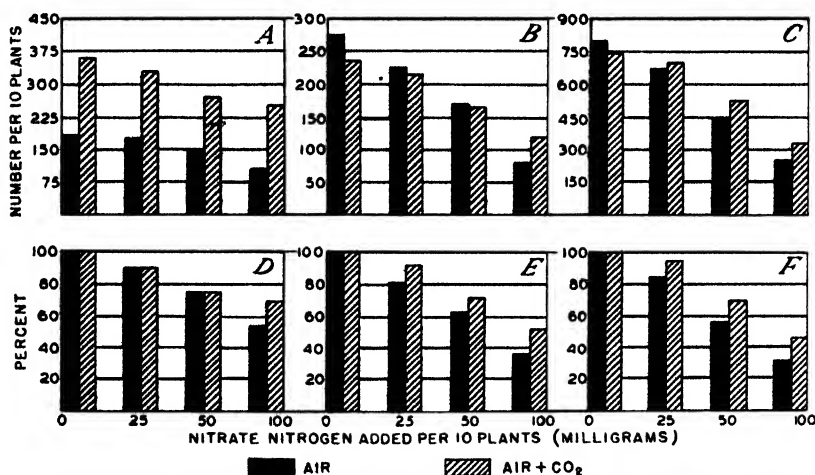


FIGURE 2.--Effect of increased $p\text{CO}_2$ on nodule production by red clover in the presence of NH_4NO_3 , 25 mg being added to each pot at the start and 25 mg weekly; experiment 2 (59 days), November 20, 1932, to January 18, 1933. A and D, total count of nodules; B and E, nodules per gram of dry weight; C and F, nodules per gram of nitrogen. In D, E, and F, the 0-mg level = 100 percent.

an increased partial pressure of CO_2 . In each part of the figure the 2 plants shown on the left were taken from the air series and the 2 on the right from the CO_2 series. The number, size, and distribution of nodules on the roots of the plants of the 2 different atmospheres and the 4- NO_3 levels are similar to those described previously and to those observed in subsequent experiments. It will be noted that the root systems are very much denser where the $p\text{CO}_2$ of the atmosphere surrounding the upper part of the plant is increased.

EXPERIMENT 3

The inorganic nitrogen source in experiment 3 was NH_4NO_3 , all of which was added at two levels of nitrate nitrogen (50 and 100 mg per 10 plants per pot) at the start of the experiment. The plants were grown during the spring of the year and were harvested when they were 50 days old. CO_2 was first passed through the CO_2 series 4 weeks after planting.

The results of this experiment were very similar to those of the preceding experiments; that is, the number of nodules decreased with

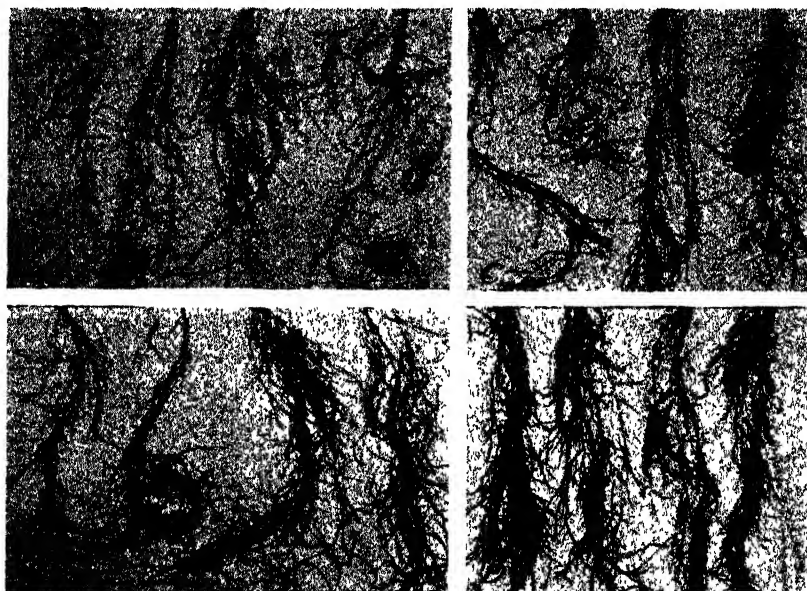


FIGURE 3.—Nodule distribution on roots of plants. These photographs are typical of plants of all four series. 0-mg level (A) and 25-mg level (B) are from experiment 2; 50-mg level (C) and 100-mg level (D) are from experiment 3. Note that the plants receiving increased $p\text{CO}_2$ present a striking degree of nodulation even though the nitrate nitrogen level has exceeded the critical concentration believed to be inhibitory under ordinary atmospheres. In each photograph plants grown in air are shown at left and those in air plus CO_2 at right.

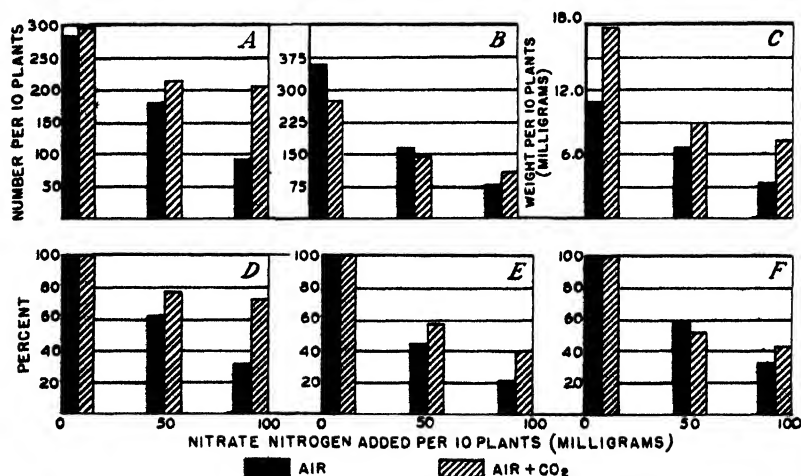


FIGURE 4.—Effect of increased $p\text{CO}_2$ on nodule production by red clover in the presence of NH_4NO_3 , which was added to all pots at the start; experiment 3, (50 days) April 7 to May 27, 1933. A and D, total count of nodules; B and E, nodules per gram of dry weight; C and F, weight of nodules per 10 plants. In D, E, and F the 0-mg level = 100 percent.

an increase in the NO_3 concentration and there was a markedly stimulating effect on nodule formation with increased $p\text{CO}_2$, with a

decided tendency for the nodules to be distributed over the secondary root system in the latter instance. Many of the plants at the 100-mg level in both series possessed no nodules, generally fewer being present on the roots of the 100-mg air series. In this experiment dry weight of nodule was determined; the data are summarized in figure 4. Both number and weight of nodule were greater on the plants given increased CO_2 .

EXPERIMENT 4

Experiment 4 was conducted during the summer months for a period of 62 days. NH_4NO_3 at three levels—0, 50, and 100 mg—was employed; 25 mg were added at the start and 25 mg weekly thereafter. Passage of CO_2 was begun 21 days after the beginning of the experiment.

TABLE 2.—Effect of increased $p\text{CO}_2$ on nodule production by red clover in the presence of NH_4NO_3 ,¹ in experiment 4 (62 days), July 5 to Sept. 5, 1933

Treatment		Plants				Nodules					
Atmosphere :	NH ₄ NO ₃	Total	Dry weight per 10	Total nitrogen per 10 :		Total count		Per gram (dry weight)		Per 100 milli-grams of nitrogen	
	Milli-grams	Number	Grams	Milli-grams	Per-cent	Number	Per-cent ⁴	Number	Per-cent ⁴	Number	Per-cent ⁴
Air -----	0	50	0.668	18.80	2.82	156	160	234	100	829.7	100
	50	65	.931	29.34	3.15	126	81	135	57.7	429.4	51.8
	100	70	.889	37.10	4.17	13	8.3	14.6	6.2	35.0	4.2
	0	50	1.024	27.82	2.72	164	100	160	100	589.5	100
CO ₂ -----	50	80	1.512	35.64	2.36	302	154	200	125	487.4	143.7
	100	84	1.845	54.25	2.94	92	56	50	31.2	169.6	28.8

¹ 25 mg. added at start; 25 mg. added weekly.

² CO_2 atmosphere contained approximately 0.15 percent CO_2 .

³ Nitrate nitrogen-free.

⁴ 0-mg level = 100-percent.

The nodule distribution was the same as in previous experiments. The complete data given in table 2 confirm the results of the other experiments in showing that with increased added combined nitrogen the percentage of nitrogen increased, the percentage of nitrogen was lower for a given treatment in the CO_2 series, and the inhibition of nodule formation both with respect to size and number was not so marked in the CO_2 series.

The plants of the 50-mg level of the CO_2 series were much larger than those of the 0-mg level of the same series. This stimulation of growth by the added combined nitrogen was accompanied by a marked increase in the number of nodules. It should be noted that with a similar increase in the size of plants (dry weight) in corresponding plants of the air series there was a decrease in the number of nodules.

EFFECT OF INCREASED $p\text{CO}_2$ ON SOLUBLE CARBOHYDRATE CONTENT OF INOCULATED CLOVER PLANTS FURNISHED COMBINED NITROGEN

The hypothesis, that the inhibitory effect of combined nitrogen on invasion of the plant and nodule development results from a decrease in the level of carbohydrate in the plant; is based on the assumption

that the combined nitrogen reduces this level by using it in protein synthesis. This assumption appears reasonable, but the necessary supporting data are meager. Strowd (18) and Orcutt and Wilson (12) have demonstrated that the addition of combined nitrogen reduces the carbohydrate content in the sap of the soybean as well as the number of nodules.

The explanation of the data obtained in experiments 1 to 4 would appear to rest on the increased carbohydrate level in the plant given added CO_2 . Five experiments were made to determine the quantitative nature of this assumed increase; only three representative ones will be discussed at length.

EXPERIMENT 5

The plants used in experiment 5 were grown during the fall of 1933. The combined nitrogen was added weekly after the first 19 days of growth in the following order for the 50-mg level: 5, 10, 7.5,

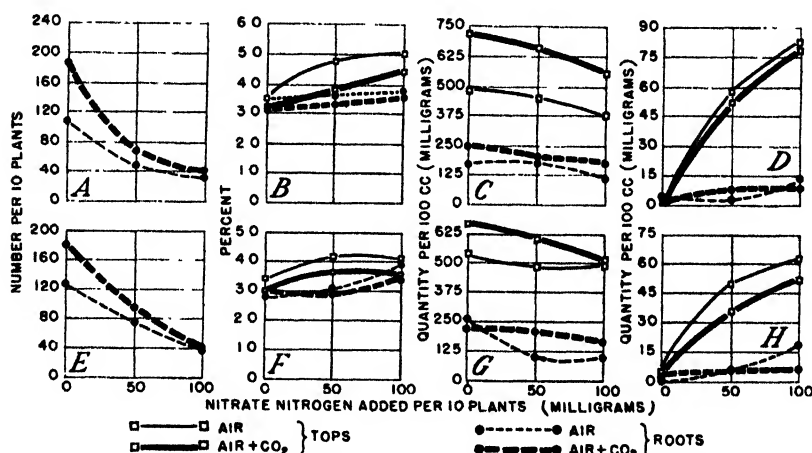


FIGURE 5.—Effect of increased pCO_2 on nodule production (A and E), percentage of nitrogen (B and F), invert sugar in tops and roots (C and G), and inorganic (NO_3) nitrogen (D and H) in red clover grown in the presence of $\text{Ca}(\text{NO}_3)_2$ in experiment 5; first harvest is shown in A to D and second harvest in E to H.

10, and 17.5 mg of $\text{Ca}(\text{NO}_3)_2$, the last amount being added on the forty-second day of growth, or 13 days before the first harvest. For the 100-mg level, there were added weekly after the first 19 days, 10, 20, 15, 20, and 35 mg per 10 plants per pot.

At the first harvest, the clover plants were 55 days old; at the second, 65 days old. CO_2 was first passed through when the plants were 21 days old, those of harvest 1, CO_2 series, being exposed to CO_2 for 34 days and those of harvest 2 for 44 days.

The inhibitory effect of $\text{Ca}(\text{NO}_3)_2$ on nodule production with the partial effectiveness of CO_2 is demonstrated in figure 5, A and E. The percentage of nitrogen is lower, generally, in the CO_2 series than in the air series; with increasing nitrate nitrogen, the percentage of nitrogen likewise increased in both series.

With increasing nitrate nitrogen concentration, the level of soluble carbohydrate tended to fall off in both roots and tops. A higher level of sugar in the sap of plants grown in increased pCO_2 is strik-

ingly illustrated. The nitrate nitrogen concentration within the plant increased but not proportionately to the quantity added to the substrate. Here the plants of the CO_2 series showed less nitrate nitrogen in the plant sap than those of the air.

EXPERIMENT 6

Experiment 6 was conducted in the spring. Half of the $\text{Ca}(\text{NO}_3)_2$ of the two nitrate levels was added at the time of planting, the rest, i. e., 25 and 50 mg, was added to the 50- and 100-mg levels, respectively, 23 days later, thereby making all of the combined nitrogen present 31 days before the first harvest.

At 54 days, harvest 1 was made, and at 61 days, harvest 2; CO_2 was started 23 days after planting.

The data in this experiment, given in figure 6, bear a marked similarity to those of experiment 5 (fig. 5). There is a tendency

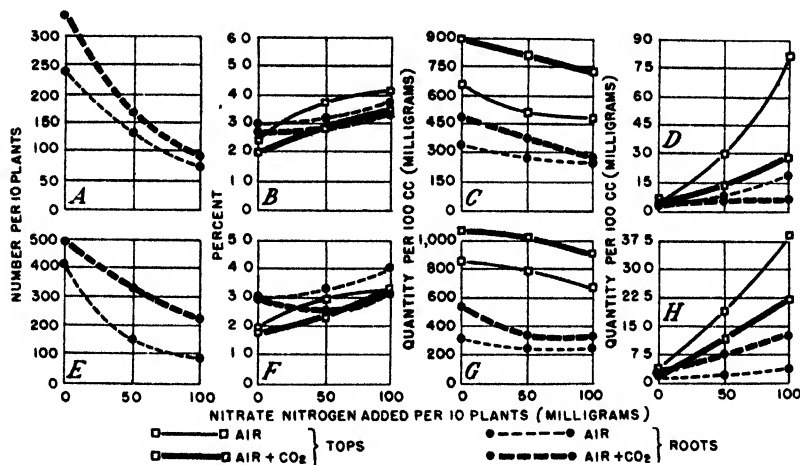


FIGURE 6.—Effect of increased pCO_2 on nodule production (A and E), percentage of nitrogen (B and F), invert sugar in tops and roots (C and G), and inorganic (NO_3) nitrogen (D and H) in red clover grown in the presence of $\text{Ca}(\text{NO}_3)_2$ in experiment 6; first harvest is shown in A to D and second harvest in E to H.

for the number of nodules to decrease at increasing levels of nitrate nitrogen, and again the CO_2 series maintains a higher margin than does the air series at the three NO_3 levels. This is more marked in the second harvest than in the first. The percentage of nitrogen increases with added combined nitrogen and is always higher in the air series for a given level of combined nitrogen. The invert sugar decreases with increasing NO_3 concentration, again a higher level being maintained by the CO_2 series.

The nitrate nitrogen in the plant sap increased with the concentration, as would be expected, analysis of the CO_2 series indicating that less existed there than in the air series. This increase was much more marked in the top portions of the plant in this experiment than in those of experiment 5. For some unknown reason, in harvest 2, the CO_2 roots possessed a greater quantity of nitrate nitrogen than the corresponding air series. This is the only instance in which such a discrepancy occurred.

EXPERIMENT 7

Because of the limited number of cellophane cans, it was necessary to employ fourteen 12-l pyrex bottles in order to carry on this experiment simultaneously with the preceding one. The technique was varied somewhat in that 20 seeds were planted per bottle and consequently the nitrate nitrogen level had to be raised in order to have nitrate present in the same proportion that it was when 10 plants per pot were used. The levels chosen were 100 and 200 mg per 20 plants per bottle. The system of growth employed was similar to that described in a previous publication (20) for 64-ounce glass bottles. Carbon dioxide determinations were made colorimetrically in small flasks suspended permanently in each bottle (16).

The experiment was carried on in the spring. The $\text{Ca}(\text{NO}_3)_2$ was added in two portions, one-half at the time of planting and the remainder at the close of 22 days.

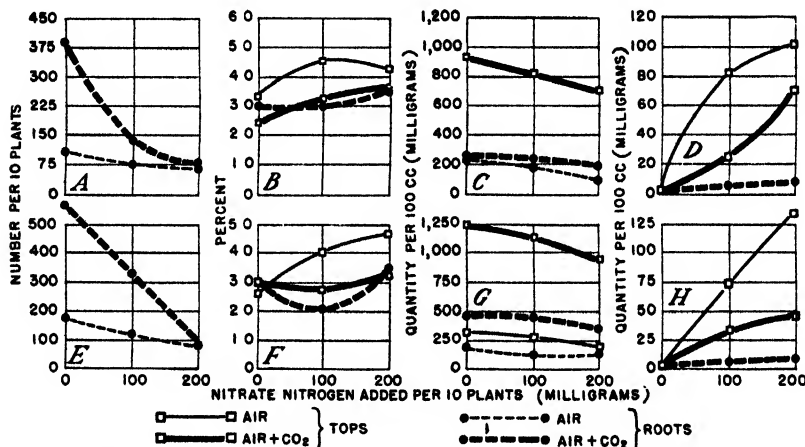


FIGURE 7.—Effect of increased $p\text{CO}_2$ on nodule production (A and E), percentage of nitrogen (B and F), invert sugar in tops and roots (C and G), and inorganic (NO_3) nitrogen (D and H) in red clover grown in the presence of $\text{Ca}(\text{NO}_3)_2$ in experiment 7; first harvest is shown in A to D and second harvest in E to H.

Harvest 1 was made when the plants were 43 days old and harvest 2 a week later. CO_2 was passed through after 22 days of growth.

The data are presented in figure 7. The general tendency is very similar to that of the preceding two experiments, except that the soluble sugars of the roots of the CO_2 series of both harvests exceed those of the tops of the air series of the same harvests. This is believed to have been caused by the lack of CO_2 in the air series. As these plants grew larger, it was found increasingly difficult to supply them with the requisite quantity of CO_2 because of the rapid assimilation, even though adjustments were frequently made to supply them at increased rates of gas flow.

The data are not complete because of the lack of root tissue in the air series of both harvests, hence there are no curves for the air roots in the percentage nitrogen or nitrate nitrogen determinations; all of the tissue was used for the carbohydrate determination and even that proved insufficient for the first harvest. The number of nodules de-

creased with increasing nitrate nitrogen concentration. This was especially true of the CO_2 series, which is contrary to the results of previous experiments. However, the nodules on the plants given increased CO_2 were much larger than those in the corresponding series given air. The percentage of nitrogen again is higher in the air series than in the CO_2 series. The soluble sugars decreased as in previous experiments, the CO_2 series maintaining a higher level in the sap of both tops and roots. The nitrate nitrogen in the sap increased with an increase of NO_3 in the substrate, the air series indicating much more present than the CO_2 series.

DISCUSSION

The quantitative data presented in this paper substitute experimental evidence for some of the assumptions on which is based the hypothesis that inorganic nitrogen reduces nodule formation in leguminous plants by decreasing the carbohydrate supply. Before discussing the interpretation of the data in relation to this hypothesis, it may be well to summarize the salient facts of these experiments.

(1) If an inoculated clover plant is furnished combined nitrogen, the number and size of the nodules decrease; with increasing quantities of nitrogen, the development of the nodules is inhibited to the vanishing point.

(2) Coincident with this antagonistic effect of combined nitrogen on nodule formation may be noted: (a) an increase in the percentage of nitrogen (narrowing of the carbohydrate-nitrogen relation in the plant); (b) a decrease in the quantity of soluble sugars in the sap; (c) an increase in the inorganic (nitrate) nitrogen.

If plants receiving combined nitrogen in quantities sufficiently large to inhibit or to suppress nodule formation are furnished an atmosphere containing increased CO_2 , the foregoing responses are modified considerably.

(3) The invasion of the plant and the development of the nodules are greatly stimulated by increased CO_2 , so that the inhibitory effects of the combined nitrogen can be partially but not completely overcome. This beneficial effect on nodule formation may be demonstrated by using the following criteria of nodule production either on an absolute or on a relative basis: (a) Number of nodules per plant, (b) number of nodules per gram dry weight of plant, (c) number of nodules per gram nitrogen of plant, (d) dry weight of nodules per plant.

(4) Accompanying the beneficial effect of increased CO_2 on nodule formation in the presence of combined nitrogen are: (a) A decrease in the percentage of nitrogen in the plants indicative of a widening of the carbohydrate-nitrogen relation obtaining therein; (b) an increase in the total soluble sugars in the sap, i. e., for a given level of combined nitrogen nutrition, plants supplied with increased CO_2 will have a higher content of carbohydrate; (c) a decrease in the level of inorganic nitrogen in the plant.

These results supply the needed experimental basis for the carbohydrate-supply hypothesis, and in a general way substantiate this explanation of the combined nitrogen effect. Qualitatively, the data show that if combined nitrogen is supplied to inoculated leguminous plants, the level of a soluble carbohydrate and the C:N relation decrease coincidentally with a decrease in nodule development. If the level of carbohydrate and the C:N relation are kept from changing in the presence of added combined nitrogen by increasing the plant's photosynthetic activities, the inhibitory effect of the combined nitrogen can be partially overcome. It seems reasonable to suppose, then, that there is an intimate interrelationship between the carbohydrate supply, the nitrogen nutrition, and the formation of nodules.

Quantitatively, the data suggest that this interrelationship is not the sole factor that conditions the response of the nodules to the presence of combined nitrogen. This is brought out in experiments

5 to 7. Although, as figures 5 to 7 show, the presence of combined nitrogen results in a decrease in the soluble sugar content of the sap, the observed decrease does not appear to be sufficiently large to account for the striking reduction in the number and size of the nodules. It is realized that the figures for the individual determinations are subject to daily and even hourly variations; hence, conclusions must be based on a fairly large number of samples. The numerous analyses made in this study as well as those on soybeans presented by Orcutt and Wilson (12) consistently show that although a definite reduction in sugar takes place in plants furnished combined nitrogen, the observed decrease does not appear to be sufficient to account completely for the accompanying reduction in number and size of the nodules.

In view of the relatively greater effect of combined nitrogen in decreasing development of nodule tissue than in decreasing the carbohydrate level in the plant sap, an explanation of the reduction of nodules by nitrates based solely on decrease in soluble carbohydrate appears inadequate. The following alternative explanations are available:

(1) The carbohydrate level in the plants, especially in the roots, is in equilibrium with the requirements of host and bacteria, and any change in the available supply results in a decrease below which normal growth cannot occur; a small decrease in the available carbohydrate may result in a large decrease in the development of nodular tissue. This view has been stressed by Allison and Ludwig (1), but no data are available that directly support the assumption. Until more definite information is obtained, judgment with respect to the adequacy of this explanation must be reserved.

(2) A second explanation that might be suggested is that the soluble carbohydrate figures are only a rough indication of the total supply of energy and building material, and that although nitrate reduces this supply quite markedly, there is a tendency for the soluble fractions to remain more or less constant. Lacking definite information with respect to which types of carbohydrates are important in this connection, the required analyses would have to include all forms—a laborious proceeding. This idea, however, can be tested in a general way by regarding the percentage of nitrogen in the plant as an inverse measure of total carbohydrate and determining whether or not plants given both CO_2 and combined nitrogen respond with respect to total carbohydrate and nodule production as do plants grown in air. To decide this question, the data from 10 experiments were combined and the correlation coefficients between number of nodules and percentage of nitrogen were determined.

TABLE 3.—Correlation coefficients between number of nodules and percentage of nitrogen

Item	<i>r</i>	<i>z</i> ¹	Proba- bility ²	<i>n</i> -3	Recipro- cal	Remarks
CO_2	-0.6896	-0.8472	<0.01	45	0.0222	} Definite negative correlation in both series; difference not significant.
Air.....	-.0666	-.7940	<.01	42	.0237	
Difference.....		0.0532±0.214		Sum.....	0.0459	

¹ For *z* test see Fisher (5, ch. 6-7).

² Probability that observed correlation arose through random sampling.

The coefficients, given in table 3, show that there is a decidedly significant inverse correlation between percentage of nitrogen and number of nodules, or a direct correlation between total carbohydrate and number of nodules. Moreover, the difference between the plants supplied with increased CO_2 and those given normal air is not significant, indicating that these two populations belong together. It is therefore concluded that, insofar as these data can supply an answer, nodule production varies directly with total carbohydrates, and the response is consistent irrespective of the treatment. It is realized that this conclusion cannot be regarded as final, since both variables (total carbohydrates and nodules) were crudely measured; possibly data concerned with various forms of carbohydrate and with weight or volume of nodules will supply a definite answer.

(3) Finally, the data are not inconsistent with the view that there is a specific effect of the combined nitrogen ion. Reference to figures 5, 6, and 7 shows that coincident with the increase in soluble carbohydrate and number of nodules, there occurred a decrease in the concentration of the NO_3 in the sap of plants given additional CO_2 . It is possible that the NO_3 exerts a specific effect in addition to or independent of its indirect effect in lowering available carbohydrate.

SUMMARY

The inhibitional effects of combined nitrogen upon nodule production by inoculated red clover plants can be partially overcome by increasing the carbohydrate synthesis in the plant; that is, by supplying inoculated plants given combined nitrogen with additional CO_2 .

Coincident with overcoming this inhibition there occurs: (1) a rise in concentration of the soluble carbohydrate in the sap, (2) a decrease in the percentage of nitrogen (widening of the C:N relation), and (3) a decrease in the concentration of combined inorganic nitrogen in the sap.

These responses are discussed with reference to their bearing on the theory that the inhibitional effects of combined nitrogen depend on the influence of the latter on available carbohydrate.

LITERATURE CITED

- (1) ALLISON, F. E., and LUDWIG, C. A.
1934. THE CAUSE OF DECREASED NODULE FORMATION ON LEGUMES SUPPLIED WITH ABUNDANT COMBINED NITROGEN. *Soil Sci.* 37: 431-443.
- (2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 3, 593 pp., illus. Washington, D. C.
- (3) BRYAN, O. C.
1922. EFFECT OF DIFFERENT REACTIONS ON THE GROWTH AND NODULE FORMATION OF SOYBEANS. *Soil Sci.* 13: 271-302, illus.
- (4) EMMERT, E. M.
1929. THE DETERMINATION OF NITRATE IN GREEN TOMATO AND LETTUCE TISSUES. *Plant Physiol.* 4: 519-528.
- (5) FISHER, R. A.
1930. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London.

- (6) FRED, E. B., and WILSON, P. W.
1934. ON PHOTOSYNTHESIS AND FREE NITROGEN ASSIMILATION BY LEGUMINOUS PLANTS. *Natl. Acad. Sci. Proc.* 20: [403]-409, illus.
- (7) HOPKINS, E. W., and FRED, E. B.
1933. INFLUENCE OF VARIOUS NITROGENOUS COMPOUNDS AND MANNITOL ON NODULE FORMATION BY CLOVER. *Plant Physiol.* 8: 141-155, illus.
- (8) ——— WILSON, P. W., and FRED, E. B.
1931. A METHOD FOR THE GROWTH OF LEGUMINOUS PLANTS UNDER BACTERIOLOGICALLY CONTROLLED CONDITIONS. *Jour. Amer. Soc. Agron.* 23: 32-40, illus.
- (9) ——— WILSON, P. W., and PETERSON, W. H.
1932. INFLUENCE OF POTASSIUM NITRATE ON NODULE FORMATION AND NITROGEN FIXATION BY CLOVER. *Plant Physiol.* 7: 597-611.
- (10) LOOMIS, W. E., APPLEMAN, C. O., PHILLIPS, T. G., TOTTINGHAM, W. E., and WILLAMAN, J. J.
1927. THE DETERMINATION OF SOLUBLE CARBOHYDRATES. *Plant Physiol.* 2: 195-204.
- (11) MAZÉ,
1898. LES MICROBES DES NODOSITES DES LEGUMINEUSES. *Ann. Inst. Pasteur* 12: 1-25, 128-155.
- (12) ORCUTT, F. S., and WILSON, P. W.
1935. THE EFFECT OF NITRATE-NITROGEN ON THE CARBOHYDRATE METABOLISM OF INOCULATED SOYBEANS. *Soil Sci.* 39: 289-290.
- (13) PHILLIPS, T. G., APPLEMAN, C. O., LOOMIS, W. E., TOTTINGHAM, W. E., and WILLAMAN, J. J.
1927. THE DETERMINATION OF NITROGEN IN RELATIVELY SIMPLE COMPOUNDS. *Plant Physiol.* 2: 205-211.
- (14) RIPPPEL, A., and KRAUSE, W.
1934. LASSEN SICH BEZIEHUNGEN ZWISCHEN KOHLENHYDRATBILDUNG UND KNÖLLCHEN BEI LEGUMINOSEN FESTSTELLUNG? *Arch. Mikrobiol.* 5: [14]-23.
- (15) SESSIONS, A. C., and SHIVE, J. W.
1928. A METHOD FOR THE DETERMINATION OF INORGANIC NITROGEN IN PLANT EXTRACTS. *Plant Physiol.* 3: 499-511, illus.
- (16) SMYTH, E. M.
1934. THE DETERMINATION OF CO₂ CONTENT OF AN ATMOSPHERE IN A CLOSED SYSTEM. *Science (n. s.)* 80: 294.
- (17) STILES, H. R., PETERSON, W. H., and FRED, E. B.
1926. A RAPID METHOD FOR THE DETERMINATION OF SUGAR IN BACTERIAL CULTURES. *Jour. Bact.* 12: 427-439.
- (18) STROWD, W. H.
1920. THE RELATION OF NITRATES TO NODULE PRODUCTION. *Soil Sci.* 10: 343-356.
- (19) WILSON, P. W.
1933. COLORIMETRIC METHOD FOR THE DETERMINATION OF CO₂ IN GAS MIXTURES. *Science (n. s.)* 78: 462-463, illus.
- (20) ——— and GEORGI, C. E.
1932. METHODS FOR CONTROLLING THE ENVIRONMENT OF GREENHOUSE PLANTS. *Bot. Gaz.* 94: 346-363, illus.
- (21) ——— ORCUTT, F. S., and PETERSON, W. H.
1932. DETERMINATION OF CO₂ IN GAS MIXTURES. A POTENTIOMETRIC METHOD. *Indus. and Engin. Chem., Analyt. Ed.* 4: 357-361, illus.

THE RELATION OF FOLIAGE COLOR TO APHID RESISTANCE IN SOME VARIETIES OF CANNING PEAS¹

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INTRODUCTION

Foliage color of canning peas varies between varieties, and occasionally within varieties, from a deep green to a light green which closely approaches yellow. As the writer has pointed out,² certain varieties of canning peas (*Pisum sativum* L.) vary widely in the degree to which they are damaged by the pea aphid (*Illinoia pisi* (Kalt.)) during seasons of severe infestation. In such years a constant relation has been observed between color of foliage and the reaction of the plant to insect injury, resistance being associated with yellow in the foliage and susceptibility with green. This paper reports the results of a study made to obtain evidence concerning this apparent relation between resistance and foliage color. A number of crosses were made between yellow- and green-foliaged varieties and the progenies studied. In this paper plants with green foliage are referred to as "green" and those with yellow foliage as "yellow."

MATERIALS

Three varieties of canning peas, Yellow Admiral, Perfection, and Onward, were used in this study. Yellow Admiral was selected because of its distinctly yellowish foliage and its observed resistance to aphid attack. This variety has a tall vine with long, slender internodes and small leaves. Perfection, which is very susceptible, is a deep-green dwarf pea with short, thick internodes and large, fleshy leaves. Onward resembles Yellow Admiral in foliage color and resistance, and Perfection in habit of growth. In 1931 these three varieties were hybridized with each other, making all possible combinations. The first generation of each cross was grown in the greenhouse during the winter of 1931-32. The second generation was grown, with woven-wire fences as supports, in the field in 1932. The entire population from each cross was grown on a separate fence, and the individuals were evaluated whenever possible as to height and color. The F₂ progeny of the Yellow Admiral-Perfection cross was separated into three classes—tall green, tall yellow, and dwarf green. The F₂ progeny of the Yellow Admiral-Onward cross also contained both tall and dwarf plants that were distinctly green, although the foliage of both parents was yellow. Therefore four classes were separated from the F₂ population of this cross—tall green, tall yellow, dwarf green, and dwarf yellow. Two classes were selected from the Onward-Perfection cross—dwarf green and dwarf yellow. Seed saved from individual plants in each class was planted in 1932 for F₃ progeny tests.

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² SEARLS, E. M. A PRELIMINARY REPORT ON THE RESISTANCE OF CERTAIN LEGUMES TO CERTAIN HOMOPTEROUS INSECTS. Jour. Econ. Ent. 25: 46-49. 1932.

It was not possible to determine the number of individuals in each class from each cross in the F_2 population. Many of the plants became infested with a virus disease which caused a chlorosis of the plants and rendered normally green plants quite indistinguishable from yellow ones.

In a subsequent cross between Yellow Admiral and Onward it was found that in the F_2 population of 164 individuals, 90 were tall green, 33 were tall yellow, 26 were dwarf green, and 15 were dwarf yellow. Dwarf is a simple recessive to tall, but the fact that yellow and green are distributed in approximately a 3:1 ratio in each of the height classes may be merely a coincidence. Green plants predominated in a cross involving two yellow strains, thereby indicating that foliage color may rest upon a complex genetic basis. Somewhat arbitrary distinctions as to color value are necessary in classifying an entire population in this way since there are at present no satisfactory methods of evaluating foliage color under field conditions. Many plants were found the color of which was intermediate between the colors of the parents. These were not used since they could not be assigned either definite or comparative values.

F_4 progenies were not used in aphid-resistance tests but were grown for increase and further elimination of lines segregating for vine height and foliage color. The F_5 progeny test was made in 1933 upon dwarf plants which had been carefully selected in the F_4 for uniformity of height and color.

PROCEDURE

In testing the F_3 populations each class from each cross was planted in a solid block. When the plants were about 3 inches high they were dusted with nicotine dust to remove any aphids that might be present and were then given a uniform aphid infestation. The aphids used for this purpose were reared on a susceptible strain of peas grown in greenhouse flats. These aphids had been brushed from the plants to the soil periodically. In crawling back to the plants they tended to distribute themselves uniformly among the plants. Aphid-laden plants were cut off at the ground and methodically placed upon the test plants.

When the plants were in full bloom a series of four aphid counts was made. This was done by bending the top 5 inches of each of 10 plants over a metal container, knocking the aphids off into the container, and counting them. At the time the counts were made many tall and green families of the F_3 were still producing dwarf and yellow plants. For this reason, only plants typical of the class in which they grew were selected for aphid counts.

Only dwarf families were used in the F_5 test. Each family was planted in 3 rows 5 feet long, 8 inches apart, and 18 inches from the next nearest family. The families were planted on August 1, 1933, in 3 groups of 14 families to each. Each group extended east and west and each family extended north and south. Plantings of parent Perfection stock were made at irregular intervals to serve as checks. The groups were lettered alphabetically, beginning at the north, while the families were numbered 1 to 14 beginning at the east. Families 1 and 14 were border families and were not used. These families were infested as heretofore except that aphids were removed from the susceptible plants by means of a small camel's-hair brush

and placed upon the leaves of the test plants. Sixteen aphids of all ages were thus placed upon each row at regular intervals.

Four aphid counts were made at 2-day intervals after the plants were in full bloom. In order to standardize the procedure the five plants growing at the south end of the eastern row of each family were cut off at the ground, all aphids were shaken from the plant into a metal container, and the aphids counted. The next five plants were taken for each succeeding count.

EXPERIMENTAL RESULTS

TABLE 1.—*Number of aphids occurring on green as compared with yellow segregates in third-generation selections, Madison, Wis., 1932*

Cross	Type of F ₂ segregate	Infestation per 10 F ₂ plants			
		Sept. 22	Sept. 24	Sept. 26	Sept. 28
Perfection × Yellow Admiral	Tall green	644	760	1,360	1,933
	Tall yellow	121	107	144	196
	Dwarf green	634	358	1,936	2,800
Onward × Yellow Admiral	Tall green	1,840	970	1,384	2,024
	Tall yellow	97	396	294	288
	Dwarf yellow	282	668	776	1,512
Perfection × Onward	Dwarf green	5,746	5,040	7,680	10,524
	Dwarf yellow	1,258	688	720	2,080

In the results of the F₃ test, given in table 1, the classes are arranged in couplets. Thus in the Yellow Admiral × Perfection hybrids the tall green class differs from the tall yellow class chiefly in color.

TABLE 2.—Number of aphids per five plants occurring on green as compared with yellow fifth-generation families from individual plant selections, and on Perfection parent stock which was used as a check, Madison, Wis., 1933

Green segregates					Yellow segregates					Perfection checks				
Family	Sept. 12	Sept. 14	Sept. 16	Sept. 18	Family	Sept. 12	Sept. 14	Sept. 16	Sept. 18	Family	Sept. 12	Sept. 14	Sept. 16	Sept. 18
A2	1,408	1,872	7,497	10,106	A3	1,552	2,412	5,206	()	A5	3,200	5,089	9,863	()
A4	2,510	4,770	11,783	()	A6	1,576	2,064	6,928	()	A9	6,400	9,281	14,981	()
A7	1,774	3,072	10,630	()	A10	1,400	2,191	6,984	()	A13	1,464	2,769	12,550	()
A8	2,208	4,353	3,650	()	A12	1,784	2,266	3,777	()	B3	2,624	4,770	6,660	()
A11	2,060	2,143	6,179	()	B4	1,606	2,266	6,872	10,377	B6	4,520	4,885	10,081	()
B2	1,476	1,120	6,783	16,393	B5	1,520	1,224	3,225	8,065	B10	1,824	4,885	9,723	()
B7	1,104	5,394	9,153	14,720	B12	1,320	2,138	3,104	8,194	C8	1,440	26,294	5,067	7,870
B8	1,456	1,823	7,042	14,600	C3	1,520	2,928	5,413	7,620	Total	21,272	26,294	69,504	7,870
B9	1,560	2,305	7,821	()	C7	260	928	1,725	4,000	Mean	3,038.9	5,258.8	9,920.1	
B11	1,552	2,031	4,352	5,563	C10	788	2,007	3,714	3,200	Mean gain		2,219.9	4,670.3	
B13	756	936	4,578	8,760	C13	624		1,125	2,944					
C2	712	1,429	1,944	10,120	Total	11,950	20,176	48,165	44,490					
C4	1,995	1,736	3,135	5,953	Mean	1,066.4	1,834.2	4,378.6	6,355.7					
C5	1,040	1,440	4,255	10,440	Mean gain		747.8	2,544.4	1,977.1					
C6	792	1,080	4,288	11,140										
C9	1,208	1,208	6,178	7,490										
C11	1,206	2,657	4,516	6,010										
C12	488	1,456	4,418	5,600										
Total	23,158	41,425	108,202	126,974										
Mean	1,286.6	2,301.4	6,011.2	9,767.2										
Mean gain		1,014.8	3,709.8	3,756.0										
Difference ¹	200.2	467.2	1,632.6	3,411.5										

¹ Dead.² Cumulative difference, greens over yellows.

As previously stated, resistance tests were not made with F_4 progenies, but F_3 progenies which appeared to be fixed for color and vine height were subjected to aphid infestation in 1933. In table 2 the infestations of the yellow progenies, the green progenies, and the Perfection check rows are listed separately, also.

In figure 1 the comparative rate of increase and number of aphids per five plants on yellow and green plants are shown. A statistical treatment of the data is given in table 3.

The data show that in all cases the green plants were more heavily infested than the comparable yellow plants. Thus in table 1 the

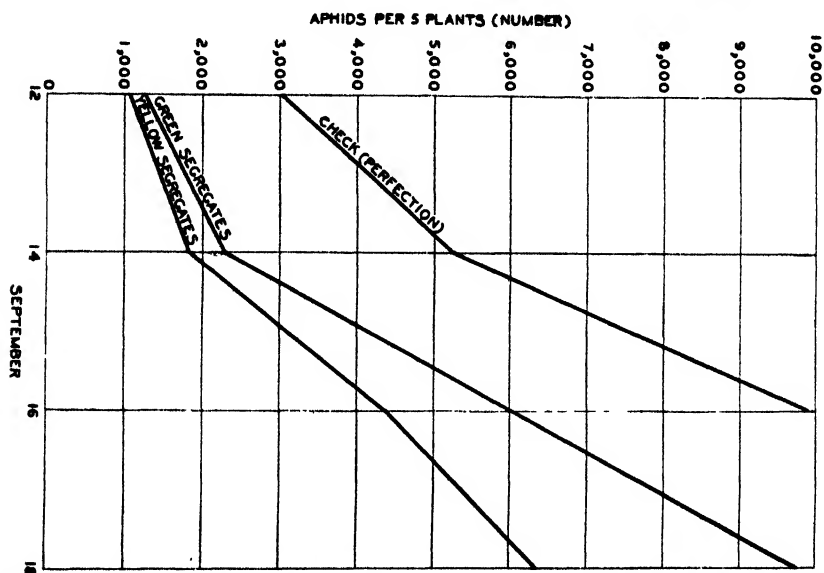


FIGURE 1.—Comparative rates of increase in aphid population on green and yellow segregates and on the green variety used as a check, Madison, Wis., 1933.

tall green class from the Yellow Admiral \times Perfection cross had about 10 times as many aphids on September 28 as the tall yellow plants. A comparable difference was observed between the tall green and the tall yellow classes from the Yellow Admiral \times Onward cross. The only direct comparison possible between dwarf green and dwarf yellow classes is in the progenies from the Perfection \times Onward cross. Here there were about 5 times as many aphids on the dwarf green plants as on the yellow at the end of the experiment. It should be noted again that only plants at the obvious extreme of either green or yellow were used in this study.

The third-generation results show clearly that resistance to aphids is heritable and is associated closely with the expression of yellow-colored foliage. Green progenies from a cross between Yellow Admiral and Onward, two yellow varieties, were as susceptible as any comparable green varieties, while the yellow progenies were resistant.

TABLE 3.—*Statistical treatment of data in table 2 showing means, standard deviation, difference between means, standard error of the difference, and ratio of latter two to each other, Madison, Wis., 1933*

Date	Green		Yellow		Mean of green minus mean of yellow	Standard error	Critical ratio
	Mean	Standard deviation	Mean	Standard deviation			
Sept. 12	1,286.6	642.4	1,086.4	547.1	200.2	223.9	
Sept. 14	2,301.4	1,294.3	1,834.2	849.6	467.2	398.4	1.17
Sept. 16	6,011.2	2,631.7	4,378.6	2,069.1	1,632.6	879.8	1.86
Sept. 18	9,767.2	3,701.3	6,355.7	2,909.8	3,411.5	1,504.4	2.27

There was considerable variation in the number of aphids which developed upon the different progenies within each class, as shown in table 2. In every case, however, the mean infestation on the green class was higher than the mean on the comparable yellow class. The acceleration of aphid numbers was also much greater on the former than on the latter (fig. 1).

Figure 1 sums up the differences in resistance between plants of the two foliage colors. All of the families dealt with mature at about the same time and, in the absence of aphids, produce satisfactory yields of peas. Owing to the absence of some factor in the green peas or the presence of some factor in the yellow peas, or vice versa, the aphids increased to lethal numbers on the green peas before they did in the yellow peas. These data indicate that in this instance the intensity of aphid population at which well developed plants were permanently wilted was about 3,000 aphids per plant. Eventually the yellow peas were destroyed also by an aphid population which appeared about as intense as that at which the green plants were destroyed, although no counts were made after September 18. This would indicate that the number of aphids which cause permanent wilting is about the same for both classes and that actual resistance rather than tolerance or avoidance is the cause of the slower acceleration of aphid numbers upon the yellow plants. The practical difference in resistance between the two hybrid groups worked with might be expressed in this difference in time in which the two groups are destroyed. If there were so few aphids that the green peas could produce a satisfactory crop before being destroyed there would be no practical difference in the expression of resistance. If the infestation were so severe that the yellow plants were also destroyed before producing satisfactorily, there would again be no practical difference. When the intensity of aphid population is such that the green peas are destroyed while yellow peas produce a satisfactory crop under comparable conditions because of the slower acceleration of aphid numbers, then there is a practical difference which may be expressed either in terms of relative acceleration of aphid numbers or in days elapsed between dates on which the critical intensity of population is reached on the different groups. This difference may be attributed to some factor for resistance in the yellow varieties. Figure 1 shows this difference. The acceleration of aphid numbers on the yellow plants was so much slower than on the green plants that many of the yellow families produced peas of canning size before they were destroyed, whereas none of the green families produced any usable peas. All of the green families had been destroyed

within a few days after the last infestation was determined. The yellow segregates lived several days longer. It is plainly evident that, with the same initial infestation, the aphids reached destructive numbers on the green plants more quickly than on the yellow plants.

Figure 1 also shows the acceleration of aphid numbers upon the check plants. Here it is quite obvious that the parent stock was even more susceptible than the segregates with apparently similarly colored foliage. The difference is not great, however. The green segregates were destroyed within a few days after the green parent.

The difference in acceleration of aphid reproduction on the two classes is again shown in table 2. Here it is plain that in each instance the yellow plants were more resistant than the green, as is evidenced by the differences in the mean gain on comparable dates, the cumulative difference in the gain in numbers on the green as compared with the yellow, and the range of aphid number on one class as compared with that on the other. The critical ratio, as shown in the statistical treatment of the data (table 3), increased each time the counts were made.

SUMMARY AND CONCLUSIONS

The object of this study was to learn more about the observed resistance of certain varieties of canning peas to the pea aphid and the apparent relation between resistance to aphid attack and color of foliage.

Three varieties of canning peas, Yellow Admiral, Onward, and Perfection were hybridized with each other making all possible combinations. Perfection, which is susceptible, has dark-green foliage while that of the resistant Yellow Admiral and Onward is a light green which closely approximates yellow. Yellow Admiral has a tall habit of growth. The other two varieties are dwarf. The offspring were classified in the F_2 as to height of vine and color of foliage. Color variation in the progenies was continuous and only those which were obviously either yellow or green were used in the subsequent tests. Third-generation families of the different classes were grown in solid blocks and artificially infested with aphids when about 3 inches high. Four aphid counts were made from each class at 2-day intervals after the plants were in full bloom. These counts showed that classes with green foliage were susceptible and that those with yellow foliage were resistant in both classes of vine height. Fifth-generation dwarf families were tested as the third generation had been, except that each family was tested separately. All the yellow families in this test were resistant while the green families were susceptible.

The tests have shown that, among the families examined, those with yellow foliage were resistant to the pea aphid while those with green foliage were susceptible. Since this relation has held through succeeding generations, it is assumed that resistance and susceptibility are inherited with the yellow and the green foliage color respectively. In every instance the plants were artificially infested with the same number of aphids. The ensuing aphid population is, therefore, an expression of ability to resist the attack of the insect rather than an instance of tolerance or avoidance.

EARLINESS OF SEXUAL REPRODUCTION IN WHEAT AS INFLUENCED BY TEMPERATURE AND LIGHT IN RELATION TO GROWTH PHASES¹

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INTRODUCTION

This paper deals with temperature and the photoperiod in relation to growth phases, earliness of sexual reproduction, and seasonal growth habit of the common wheat plant. In addition data are presented on the effects of two types of electric light used to lengthen the natural day in relation to the time of sexual reproduction and the number of seeds produced.

Part of the data here presented support portions of a note (25)² and of a previous paper (27) by the writers.

REVIEW OF LITERATURE

So far as the writers are aware, Adams (2) was the first to point out the importance of temperature in relation to the daily photoperiod in regulating the time of sexual reproduction in wheat. He claimed that these two factors are interchangeable. While the results of his tests suggest a compensatory relationship, they do not prove the point, since temperature was not varied concurrently with the length of the daily photoperiod. He obtained his different temperatures by conducting the tests during different periods of the year. The following year Adams (3) continued his study of temperature and the photoperiod, but he carried out no experiments with wheat that gave evidence on the interchangeable relationship between temperature and the photoperiod.

Enomoto (9) conducted experiments that showed the importance of both temperature and the photoperiod in relation to earliness in wheat and barley. The writers (25) presented brief data on temperature and the photoperiod and indicated their compensating relationship with respect to earliness in wheat. Maximov (31) indicated the same relationship with respect to wheat in a statement, but he presented no data. Hurd-Karrer (19) has shown the influence of temperature and the photoperiod on a spring wheat and of the photoperiod on a winter wheat. Several workers have studied temperature (17, 21, 22) and the photoperiod (8, 10, 13, 30, 43) independently in relation to the development of the wheat plant.

The majority of the environmental studies that have been conducted under controlled conditions have been managed so as to maintain as nearly constant conditions as possible throughout all the growth

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² Reference is made by number (italic) to Literature Cited, p. 639.

phases of the plant, little or no attention being given to special requirements of the plant at different stages of development. This is the orthodox method, and while it is reasonably satisfactory for the study of certain types of plants such as the spring cereals, it is not satisfactory for many important studies on such plants as the winter cereals.

The literature shows that the temperature relations of the growth phases in winter cereals, especially the stimulating influence of low temperature during early germination on subsequent earliness in winter wheat, were recognized many years ago. However, this knowledge was not widely accepted, and it appears that these relationships have been lost sight of and rediscovered several times since the first record.

The writers (27) pointed out that Klippart (23, p. 757) knew before 1857 that low temperatures applied to germinated seed induced winter wheat when sown in the spring to mature and produce a good crop. Recently Martin³ pointed out that this phenomenon was noted in winter wheat as early as 1837 by a grower in New York (1), but as irregular heading was reported by this grower it is apparent that the time of exposure to low temperatures was too short or that germination was incomplete during the period of low temperatures. In 1850 Allen (4, p. 128) made a similar report in his book, but he thought germination unnecessary before chilling. He was not aware that germination had started in the soaked seeds held near freezing. Klippart's report indicates that he had a better understanding of the importance of germination and of the exposure time than did those before him, but he and those before him did not seem to understand that freezing temperatures are less effective (27) than temperatures slightly above the freezing point. Recently this and other methods of accelerating sexual reproduction have been designated in Russia by the term "iarovization", meaning vernalization, the English equivalent. Literature and experiments on this subject are dealt with in other papers⁴ (6, 26, 28, 44).

Helbrigal (16), working with barley advanced beyond the early germination stage, concluded that this plant has a lower optimum temperature during the tiller-formation phase than during the stem-elongation phase. Gassner (15), Maximov and Pojarkova (33), Pojarkova (37), and Papadakis (35) concluded from experimental data that earliness in winter wheat is favored when low temperatures obtain during the seedling stage and when higher temperatures obtain during the later stages of growth.

In studies on the photoperiod Wanser (43) concluded that the several growth phases of the plant have different length-of-day requirements. Rasumov (39) studied the influence of short days followed by long days, long days followed by short days, continued short days, and continued long days on earliness in millet, spring oats, and spring barley, and he conducted field plantings with spring wheats. No tests with winter wheat were reported. With millet Rasumov found earliness of sexual reproduction favored equally by the continued short day and by 10 short days followed by 6 long days. His data on oats and barley show that the constant long days

³ MARTIN, J. H. IAROVIZATION IN FIELD PRACTICE. U. S. Dept. Agr., Bur. Plant Indus. 13 pp. 1934. [Mimeographed.]

⁴ ——— Martin, J. H. See footnote 3.

avored earliness. A test carried out by Hurd-Karrer (18) indicated that a short day followed by a long day favored early jointing in Turkey winter wheat. The writers (25) presented preliminary data showing that early sexual reproduction in winter wheat is favored by an initial exposure to short days followed by long days. Maximov (31) drew the same conclusion but presented no data. In a later paper summarizing work on temperature and the photoperiod, Maximov (32) erroneously stated that the writers (27) found that shortening the day at the higher temperatures during the first week of growth made it possible for winter cereals to fruit the first year. The writers' studies indicate that an exposure of but 1 week is too short to induce more than an exceedingly slight acceleration of earliness in the winter wheats studied. Forster et al. (10) conclude that an initial short day hastens maturity in winter wheats.

In addition to the environmental studies made under controlled or semicontrolled conditions, there have been a number of studies based on date-of-seeding tests in the field, a few of which are cited (5, 11, 14, 24, 36).

METHODS AND TERMINOLOGY

The experiments were conducted during the winter and spring seasons in culture chambers or in greenhouses, and some tests were conducted outdoors during the summer. The plants were grown in good soil in earthen pots 8 inches in diameter. Each pot supported two plants. Care was exercised in maintaining uniform soil moisture throughout a given test by frequent applications of water.

In certain tests the seeds of Harvest Queen winter wheat were germinated and chilled at 26° to 32° F. in a dark mechanical refrigerator for several weeks before planting, as indicated.

Days longer than the natural day were obtained by means of electric lamps turned on at sunset. Except when otherwise stated, the electric source consisted of Mazda C tungsten lamps which gave an intensity ranging from 20 to 40-foot candles at the soil line. The length of the natural day was taken as from midday to midtwilight, an increase of 1½ to 1¾ hours over a natural day length taken as from sunrise to sunset. Days longer than the natural day were computed from midday. The 8-hour day started at 8 a. m. and ended at 4 p. m.

The initial growth phase of winter wheat, which, under cultural conditions, takes place in the autumn and winter, has commonly been referred to as the rosette phase or as the resting or dormant stage. However, it has been pointed out that this is not always a true resting stage and that a resting or a dormant condition during early growth is not essential for sexual reproduction in winter wheat (10, 22, 42). These conclusions are borne out by the writers' studies. In regions of very cold winters the plants are dormant during the winter period, but at Rosslyn, Va. (near Washington, D. C.), growth continues slowly throughout the winter except in an occasional year when there may be a short dormant period. In this paper this stage is referred to as the "leaf-and-internode-formation phase", and the term is applied to winter and spring varieties. Hellriegel (16) used a similar terminology for barley. The so-called jointing stage is designated the "stem-elongation phase."

All data on earliness are based on the date when the tip of the head reached a point as high as the ligule of the flag leaf. Fertilization occurred 2 to 4 days after heading.

Further details on methods are taken up throughout the paper under the several experiments.

EXPERIMENTAL RESULTS

TEMPERATURE AND THE PHOTOPERIOD IN RELATION TO THE GROWTH PHASES AND SEXUAL REPRODUCTION

During the winter of 1928-29 a test was conducted with Harvest Queen, a typical winter wheat. Plantings were made in a greenhouse with germinated seeds that had been held in a refrigerator for 67 days at 30° to 35° F. Unchilled germinated seeds were planted as controls. Some of the plants were exposed to the natural day, which ranged from 11 hours at the beginning of the test to 15 hours at the end, and others to a long day, ranging from 16 to 17 hours. Mazda C tungsten lamps were used for supplementing the natural day. The temperature of the greenhouse was usually about 55° to 65° F., though on bright days the temperatures went higher.⁵

Table 1 and figure 1 show that of the plants from the chilled seedlings, those under the long day headed first; of the plants from the unchilled seedling, those under the short day headed first; under both the long and the short day, the plants from the chilled seedlings headed earlier than the plants from the unchilled seedlings. This test shows the stimulating influence of low temperatures during early germination, in the absence of light, on subsequent earliness of sexual reproduction in long days with high temperatures.

TABLE 1.—*Period from planting to first heading in Harvest Queen wheat as influenced by chilling in darkness during germination and by the length of the photoperiod after chilling in comparison with plants from seed not chilled before planting*

[Seed was planted Dec. 18, 1928]

Length of daily photoperiod after planting	Germination temperature 30° to 35° F. for 67 days in darkness, followed by 55° to 65°		Germination temperature 70° F. for 4 days in darkness, followed by 55° to 65°	
	Period from planting to heading	Tillers per plant when internodes started to elongate	Period from planting to heading	Tillers per plant when internodes started to elongate
	Days	Number	Days	Number
11 to 15	118	5.1	125	12.2
16 to 17	66	2.9	128	12.9

Since the length of day in the short-day series was 4 hours shorter during early growth than during later development, it seemed probable that this initial short day was a factor in the earlier heading noted in the plants from the unchilled seedlings. The following winter this relationship was studied further with Harvest Queen winter wheat and Purplestraw, a facultative wheat.⁶ As stated in a note (25), a 7-

⁵ This statement was inadvertently omitted in a previous publication (#7).

⁶ The writers have discussed the basis of distinction between spring, facultative, and winter varieties of wheat in another publication (#7).

to 8-hour day during the early development of the plant favored earliness slightly in Harvest Queen, but this short day retarded heading slightly in Purplestraw.

During the same winter another test was carried out with Harvest Queen wheat and with Marquis, a spring wheat. The seeds were germinated but not chilled before planting. Some of the plants of



FIGURE 1.—Harvest Queen winter-wheat plants: *A* and *C*, From germinated seeds chilled at 30° to 35° F. in the dark for 67 days; *B* and *D*, from seeds that were not chilled; *A* and *B*, grown with a daily photoperiod of 11 to 15 hours; *C* and *D*, grown with a daily photoperiod of 16 to 17 hours.

each variety were grown at low temperatures in plant-culture chambers during the first 54 days, and later they were given high growing temperatures in a greenhouse. Other plants of each variety were grown at high temperatures in a greenhouse throughout the test. Short, medium, and long days were maintained during the first 54 days at the low and high temperatures (table 2).

Table 2 and figures 2 and 3 show that the longest days at the high temperatures from the beginning of growth induced the earliest heading in Marquis spring wheat (46 days) and the latest heading in Harvest Queen winter wheat (155 days). The shortest days at the low temperatures during the first 54 days induced the earliest heading in



FIGURE 2.—Marquis spring-wheat plants (table 2): *A*, *B*, and *C*, Grown in a greenhouse at temperatures averaging near 70° to 75° F. and above; *D*, *E*, and *F*, grown in a culture chamber near 51° for 36 days, followed by temperatures near 59° for 18 days, followed by 70° to 75° and higher; *A* and *D*, with daily photoperiod 8 hours long for first 54 days; *B* and *E*, with daily photoperiods 12 to 14¼ hours long for first 54 days; *C* and *F*, with daily photoperiods 16¼ to 17¾ hours long for first 54 days; subsequent daily photoperiods, 17¾ to 19 hours.

Harvest Queen (88 days) and the latest heading in Marquis (88 days). Fertilization started 2 to 4 days after heading in both varieties. These results, like those of Garner and Allard (13), indicate that Marquis spring wheat is a long-day plant with respect to earliness of sexual reproduction.

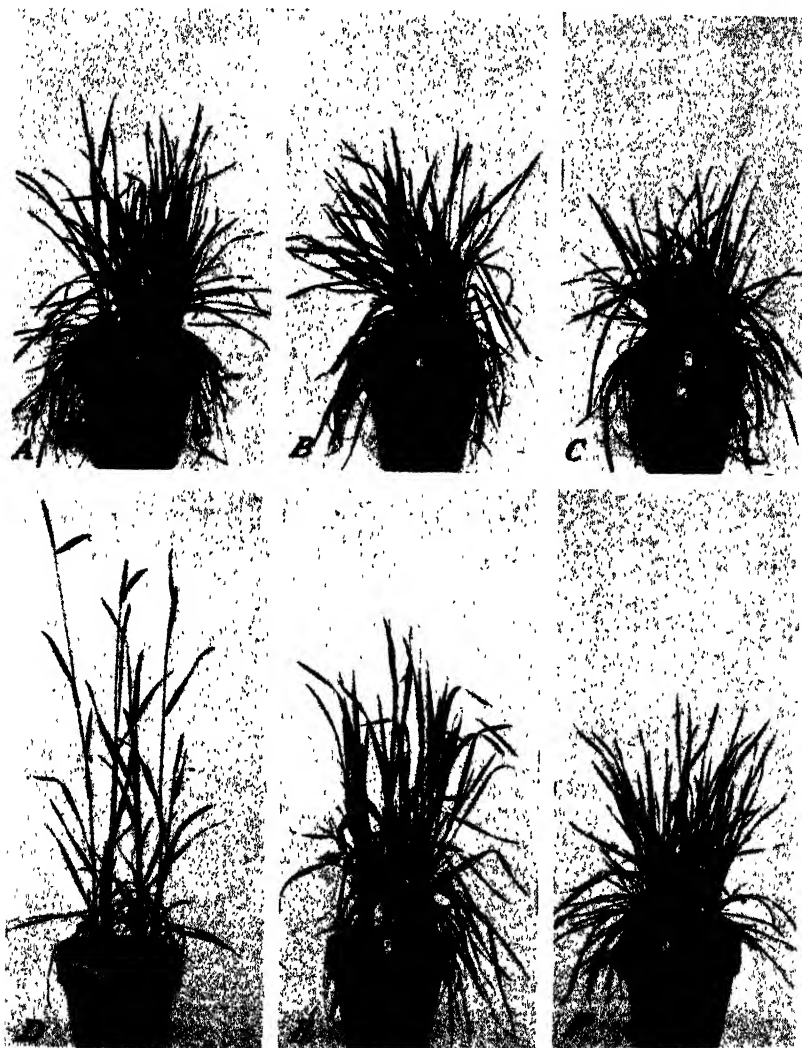


FIGURE 3.—Harvest Queen winter-wheat plants (table 2): *A*, *B*, and *C*, Grown in a greenhouse at temperatures averaging near 70° to 75° F. and above; *D*, *E*, and *F*, grown in a culture chamber near 51° for 36 days, followed by temperatures near 59° for 18 days, followed by 70° to 75° and higher; *A* and *D*, with daily photoperiod 8 hours long for first 54 days; *B* and *E*, with daily photoperiods 12 to 14¼ hours long for first 54 days; *C* and *F*, with daily photoperiods 16¼ to 17¾ hours long for first 54 days; subsequent daily photoperiods, 17¾ to 19 hours.

It is evident from the data and the illustrations that the short days and the low temperatures during early growth (the leaf-and-internode-formation phase), followed by the longer days and higher temperatures, favored the earliest sexual reproduction in the winter variety,

thus indicating that the early growth phase has different temperature and photoperiodic optima from those of the subsequent growth phases.

TABLE 2.—*Heading time in Marquis spring wheat and Harvest Queen winter wheat as influenced by low and high growing temperatures and by short, medium, and long daily photoperiods during the first 54 days, after which all plants received high temperatures and long days as indicated*

[Seeds were soaked and germinated for 2 days before being planted Feb. 12, 1930]

Variety and photoperiod ¹	Temperature, 51° F. for 36 days; then 59° for 18 days; subsequently 70° to 75° and above			Temperature, 70° to 75° F. for 54 days; subsequently 70° to 75° and above		
	Period from soaking of seed to heading	Tillers per plant	Heads per plant	Period from soaking of seed to heading	Tillers per plant	Heads per plant
	Days	Number	Number	Days	Number	Number
Marquis:						
8 hours.....	88	14.5	8.0	78	8.5	4.0
12 to 14½ hours.....	83	5.5	6.2	76	12.5	4.0
16¼ to 17¾ hours.....	65	8.2	7.2	46	4.5	4.0
Harvest Queen:						
8 hours.....	88	17.7	4.2	* 107	19.2	4.7
12 to 14½ hours.....	95	22.5	5.2	* 134	21.2	2.0
16¼ to 17¾ hours.....	* 114	22.5	4.7	* 155	22.5	3.0

¹ After 54 days all daily photoperiods were maintained at 17¾ to 19 hours.

² Many of the tillers on these plants did not head, but this condition was less marked than in the plants designated in footnote 3.

³ All plants were very vegetative and heading was slow, requiring 50 to 60 days for completion in the case of some plants. On Aug. 15 some plants still were producing tillers that were jointing and heading.

It was not possible to include simultaneously a series of temperatures and photoperiods held constant or nearly so during the life cycle to serve as additional controls in this experiment. However, other tests have been carried out with Harvest Queen and similar winter wheats grown constantly near 60° F. and also at temperatures between 60° and 75° in constant long days and in constant short days. In all instances, more than 100 days to more than 200 days elapsed from planting to heading and fertilization. Tests of Hutcheson and Quantz (21), Klages (22), and Hurd-Karrer (17, 19) also show that heading in winter wheat is relatively slow when the temperature requirements of the growth phases are disregarded.

An experiment was conducted with Harvest Queen winter wheat during midsummer with daily photoperiods of 8, 15, 18, and 24 hours. Each photoperiod remained unchanged throughout the test. The 8- and 15-hour days comprised sunlight only, whereas the 18- and 24-hour days comprised sunlight for 15 hours and electric light from Mazda C tungsten lamps for the remainder of the photoperiod. The seeds were soaked in water and germinated before planting, but they received no low temperatures. The plants given 18 and 24 hours of light daily headed 129 and 110 days, respectively, from the time the seeds were placed in water. No heads were produced by plants given 8 and 15 hours of light daily up to the time the test was terminated on November 11—178 days from the beginning of the test.

These results by themselves would lead to the conclusion that winter wheat is a typical long-day plant with respect to early sexual reproduction, as was concluded by Forster et al. (10) from similar

tests, and by Hurd-Karrer and Dickson (20) from tests (19) conducted in a cool greenhouse during the winter. However, in table 2 it will be noted that Harvest Queen headed in 88 days when the plants were grown with a daily photoperiod of 8 hours at low temperatures followed by long photoperiods at high temperatures. Thus heading was 22 days earlier with this treatment than it was when continuous light and high summer temperatures obtained from germination to heading.

Under the conditions of Hurd-Karrer's (19) test, flowering started in Turkey winter wheat 188, 165, and 132 days from planting when grown with daily photoperiods of 8, 9½ to 15, and 17 hours, respectively, and with the temperature held at $12^{\circ} \pm 1^{\circ}$ C. throughout the test. With the same strain of Turkey the writers (27) found that flowering started 113 days from planting when moderately low temperatures and short days preceded higher temperatures and the long day. In later tests, this strain of Turkey started to flower 105 days after planting, when the temperatures and exposure periods were nearer their optima. Other varieties of winter wheat have been tested and found to head and flower sooner when suitable low temperatures and short days were followed by suitable high temperatures and long days.

The results of more than 500 dissections of many varieties of winter and spring wheats indicate that the first signs of differentiation of the floral organs become evident soon after the last leaf primordium is differentiated, and that the internodes of the stem start their major elongation a little before or simultaneously with the differentiation of the floral organs.

The number of leaves and stem internodes formed by a shoot in winter wheat as well as in spring wheat (27) is influenced by temperature and the photoperiod. Harvest Queen wheat has produced as few as 7 internodes' per shoot when fully germinated seeds were first chilled for 67 days in darkness and subsequently grown with continuous light at summer temperatures. Heading started 33 days after the completion of the chilling process. As many as 22 internodes per shoot have been produced by this variety when the seed was not chilled and when the plants were grown in uninterrupted light at warm temperatures, and under these conditions heading started 110 days after planting. Similar results were obtained when a continuous 8-hour day and warm temperatures were maintained from the beginning of germination. In field culture Harvest Queen has been observed to form 12 or 13 leaves and internodes on the primary stalk. When small numbers of internodes are formed most of them elongate, but when large numbers are formed many fail to elongate appreciably. The early cessation of the formation of leaf primordia in Harvest Queen wheat does not take place during the exposure to the low growing temperatures and short days, but during the subsequent exposure to the high temperatures and long days.

To classify accurately varieties of wheat with respect to their earliest sexual reproduction in relation to temperature and photoperiod it is necessary to determine the optimum temperatures and

optimum photoperiods for the formation of a reduced number of stem-internode primordia, the early differentiation of the floral primordia, the rapid elongation of the internodes, and the rapid development of the floral organs and maturity. It seems reasonable to believe that ultimately a graphic representation of the optimum temperature and photoperiod characteristic of a given variety of winter wheat can be expressed in the form of more or less smooth gradient curves. However, until more perfect methods are available, approximations must suffice.

In view of the evidence based on present methods for approximating these optima, it seems justifiable to conclude that Harvest Queen, Turkey, and similar winter wheats are not typical long-day plants with respect to their earliest sexual reproduction, but are what may be termed short-day→long-day plants, and they may be considered as low-temperature→high-temperature plants. This method of expression indicates that the temperature and the photoperiod must increase with the development of the plant in order to induce early sexual reproduction. A similar situation seems to apply to the facultative varieties and to certain late varieties commonly placed in the spring group (27), but in these the initial optimum temperatures are higher or the periods of exposure to low temperatures are shorter than is the case with the strictly winter varieties (27).

None of the above tests was planned for yield determinations. However, tiller counts are recorded in tables 1 and 2 and head counts in table 2. Conditions favoring earliness favored a reduction in the number of tillers. Observations indicated that Marquis produced larger and more completely filled heads when the initial temperatures were low. In Harvest Queen the plants receiving the initial low temperatures with the initial 8-hour day produced an average of 75 seeds per plant, and these plants were similar to the average Harvest Queen plant grown in the field. Although no seed counts were made on the remaining Harvest Queen plants, it appeared from observations that the greatest number of seeds was produced by the plants receiving the initial low temperatures with the medium length of day, and these plants were considered to exceed the normal. The continuous high temperature and the initial long day at the initial low temperatures favored a large number of tillers in Harvest Queen, but the few heads produced were relatively small and poorly filled and the plants were decidedly abnormal in all respects in comparison with the field type.

MISCELLANEOUS TESTS RELATING TO CHILLING OF SLIGHTLY GERMINATED SEED

DARKNESS V. LIGHT DURING CHILLING PERIOD

An experiment was carried out to determine if darkness during the chilling treatment has any stimulating influence on subsequent earliness. Germinated seeds of Turkey winter wheat were chilled for 61 days at 38° F. Some of the seeds were in total darkness and others received daylight. After chilling, growth continued outdoors at summer temperatures with a daily photoperiod of 17 to 18 hours. The plants from seed chilled in daylight headed 47 days after planting, and those from seed chilled in darkness headed in 49 days. From these results it seems evident that the low temperature is the stimulating agent for subsequent early heading.

IMPORTANCE OF MOISTURE AND GERMINATION DURING CHILLING PERIOD

An experiment was carried out with dry and with moist ungerminated seeds, with seeds that were germinated and then dried and with seeds that were germinated and kept moist during the process of chilling. All seeds were chilled for 65 days near 34° F. After being chilled the seeds were planted with an unchilled control at temperatures near 70° to 75° and photoperiods that ranged from 16 to 18 hours.

Plants from dry seeds, whether germinated or not, and from unchilled seeds had produced no heads when the test was discontinued 150 days after planting. Plants from seeds that were germinated and kept moist during the chilling process headed in 45 days. The plumules and radicles of seeds kept moist but not germinated before the beginning of the chilling process were showing slight activity at the end of the chilling treatment. Plants from these seeds started to head in 135 days, but heading was irregular.

STAGE OF DEVELOPMENT OF THE SEED

A test was carried out with Harvest Queen seed in the soft-dough stage and in the hard-dough stage and with completely ripened seed 1 year old, to determine whether low temperatures during germination stimulate subsequent early heading in plants from seeds in the soft-dough and in the hard-dough stage. All seeds were first germinated at 60° F. and then chilled near freezing for 72 days. After chilling, growth continued at 68° to 90° with a daily photoperiod of 16 to 17½ hours. Plants from seed in the soft-dough stage headed in 43 days, those from seed in the hard-dough stage headed in 45 days, and those from the old seed headed 43 days from the date of planting, thus showing that seed in the soft-dough and hard-dough stages reacts efficiently to the chilling treatment. This permits a more rapid increase of winter-wheat populations in genetic work.

EARLINESS AND TILLER REDUCTION

When germinated seeds have been chilled sufficiently, several tillers ultimately joint and produce heads relatively quickly at suitable temperatures and day lengths, thus indicating that the function altered by the low temperature is not confined entirely to the plumule (primary shoot).

It was thought that the effects of chilling on early stem elongation and heading at high temperatures might be due directly to the reduction in the number of tillers, which is correlated with the early heading of plants from chilled seedlings. If this were true, another method of reducing tillers might accelerate stem elongation and heading. To test this theory, several Harvest Queen plants from unchilled seedlings were grown outdoors during the summer of 1931. Some of these plants were allowed to grow in the usual manner, whereas others were not allowed to produce more than one shoot each, all extra shoots being cut from the plants as soon as observed. The single shoots developed very long, wide dark-green leaves and very large stems, in comparison with the unpruned plants. However, the fact that pruning did not stimulate early heading indicates that the reduced tillering in plants from chilled seedlings is not the direct cause of early heading.

LOCALIZATION OF THE REGION OF THE FUNCTIONAL CHANGES INDUCED BY LOW TEMPERATURES

In an attempt to localize the region in which the functional changes occur at low temperatures, seeds of Kanred winter wheat were germinated until the roots were 2 mm long; the roots and plumules were then cut off before the initiation of the chilling process, care being taken to remove the tips and as much of these organs as possible without total destruction of the embryo. These and unmutated seedlings were chilled near freezing in the dark for 65 days. At the completion of the process the seedlings were given continuous light at 70° to 80° F. All the mutilated seedlings headed at the same time as the unmutated controls. It is evident, therefore, that the active region in question is not confined to the apical regions of the seminal roots, the coleoptile, or the first leaf. It is also apparent that this active region is not confined to the endosperm, because under field conditions at the Arlington Farm the endosperm frequently is completely absorbed in early autumn before the onset of cold weather, and under these conditions the winter wheat plants mature normally the following summer.

INFLUENCE OF INTENSITY OF LIGHT FROM TUNGSTEN LAMP

Since the Mazda tungsten lamp is commonly used for lengthening the natural day, a test was conducted to determine the influence of intensity of this artificial source of light during the winter and spring months. Marquis, Siberian No. 1, and Romanov spring wheats were used. Each variety was grown in earthen pots 8 inches in diameter, each pot containing two plants. The pots were placed in 3 parallel rows 12 feet long on a bench in a small glasshouse, pots containing plants of the same variety being placed in the same row.

Two 150-watt Mazda C tungsten lamps in a large reflector were placed at one end of the bench but not directly over the first pots. The plants farthest from the lamps, therefore, received less light at night than those nearest the lamps. The lamps were turned on at sundown and off at sunrise each day during the test, after which daylight only was provided, giving the plants a continuous exposure to light.

The intensity of the light from the lamps was determined during the night at three distances above each pot in the center row, as indicated in table 3. The test plate of a Macbeth illuminometer was held at right angles to the shortest distance between the light source and the center of the plate. Owing to the shape of the lamp reflector and to shadows from intervening plants, the intensities reduced more rapidly than is provided for in the inverse-square-distance law.

Care was exercised in maintaining a uniform temperature near 70° F. throughout the experiment by means of oscillating fans. As spring advanced there was a rise in temperature outdoors, but the uniformity of the temperature was maintained throughout the greenhouse.

TABLE 3.—*Influence of intensity of Mazda C tungsten lamps on 3 varieties of wheat when grown near 70° F. during the short days of winter, the tungsten source being used from sunset to sunrise, making continuous illumination*

[Test started Dec. 8, 1931]

Pot no.	Intensity of lamps at indicated heights above soil line over each pot in center row			Heading time			Culms per plant			Average final height of plants		
	4 inches ¹	18 inches ²	28 inches ³	Siberian No. 1	Marquis	Romanov	Siberian No. 1	Marquis	Romanov	Siberian No. 1	Marquis	Romanov
	Foot-candles	Foot-candles	Foot-candles	Days	Days	Days	Number	Number	Number	Inches	Inches	Inches
1.....	76.3	100.0	272.5	39	48	50	3	8	6	20	26	31
2.....	56.7	63.0	93.7	42	51	50	4	14	12	22	28	31
3.....	38.1	25.0	38.1	43	51	51	5	10	9	21	26	31
4.....	25.1	12.0	22.9	42	51	52	5	11	7	21	27	31
5.....	20.0	9.0	15.3	43	53	53	4	10	9	23	23	34
6.....	8.7	5.0	7.0	45	52	56	5	9	12	17	28	29
7.....	6.8	4.3	5.4	45	53	53	4	10	12	19	28	37
8.....	5.8	2.0	4.0	50	63	63	6	18	11	22	28	37
9.....	4.5	1.2	3.4	50	64	64	8	11	12	27	25	39
10.....	3.6	.7	2.8	52	69	69	9	8	8	21	31	42
11.....	3.1	.65	2.4	59	75	76	16	10	7	27	32	43
12.....	2.5	.5	2.0	69	91	91	14	11	10	34	33	45

¹ When this reading was made the plants did not cast a shadow on adjacent plants.

² When this reading was made the plants ranged from 20 to 28 inches tall, and the test plate was shaded by adjacent plants except in the case of the first plant in the row.

³ When this reading was made the test plate was not shaded by adjacent plants.

The data in table 3 show that earliness was favored by the more intense light. In general, the differences in response of the plants became most evident when the light intensity at 28 inches above the soil line during the night period reached two foot-candles and less. Siberian No. 1 produced the least number of tillers near the lamps and the greatest number when at the greatest distance from the lamps. Tillering in the other varieties did not vary appreciably as the light intensity was varied. The final height of the plants was greatest in plants farthest from the lamps. Romanov, with slight exceptions, showed the most consistent gradual increase in height as the light intensity was reduced.

Leaf and straw sizes were greatest when the supplementing light intensity was reduced. The leaves averaged five-sixteenths of an inch wide by 11 inches long near the lamps and one-half of an inch wide by 18 inches long farthest from the lamps. The largest nodes on plants near the lamps averaged 0.107 inch in diameter, whereas those of plants growing farthest from the lamps averaged 0.175 inch in diameter.

Romanov and Marquis heads did not fill well, whereas Siberian No. 1 filled well in all intensities of light. Many heads on Marquis plants contained but two or three kernels.

The most rapid stem elongation occurred in the plants that were first to head (fig. 4), but the plants that headed last continued their growth for a longer period and at maturity were much taller than the ones that headed first (table 3).

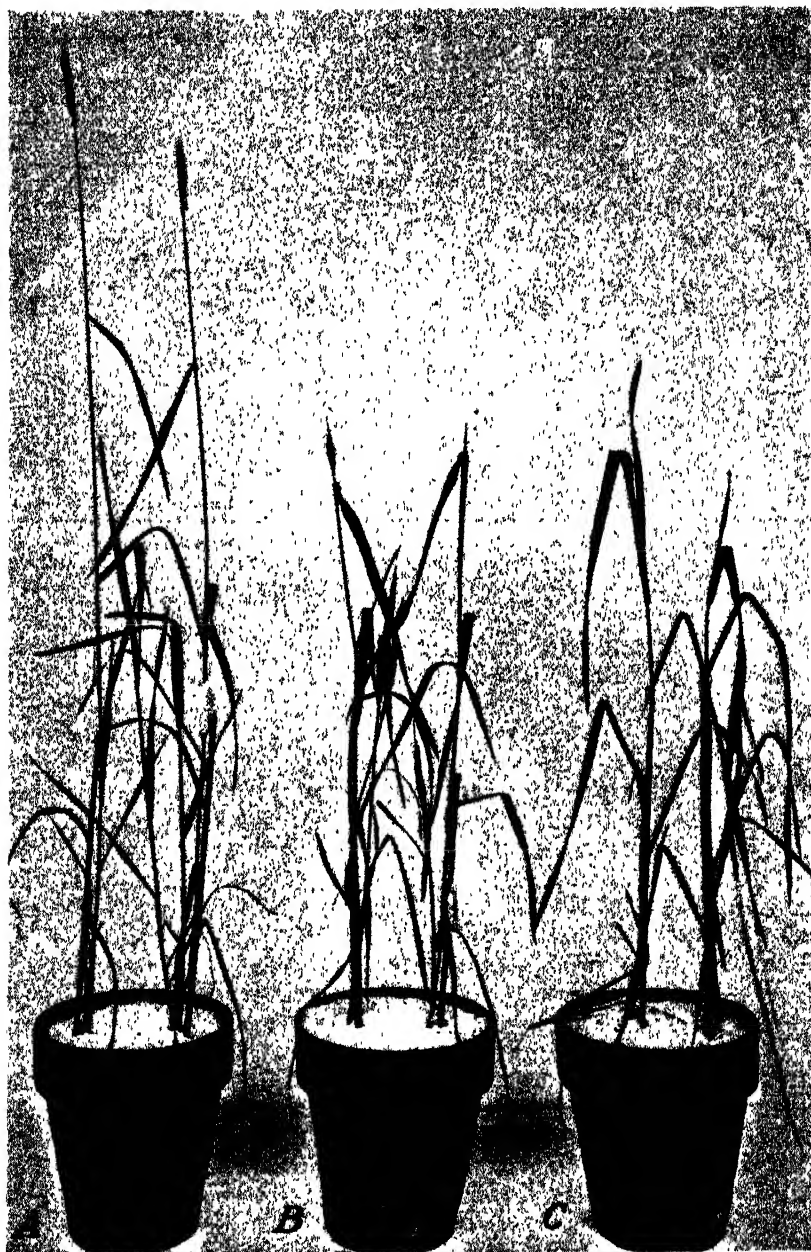


FIGURE 4.—Romanov spring wheat grown near 70° F. with uninterrupted light during the winter and early spring months. Mazda C tungsten lamps were turned on at sunset and off at sunrise; during the day all plants received equal intensities of sunlight; during the night different plants received different intensities of light. Pots A, B, and C correspond to pots 1, 6, and 12 in table 3.

Although the intensity of the supplementing light influences earliness and other responses of the plant, the data presented indicate that changes did not become marked until the light intensity had changed to a relatively great extent. This is borne out also in Garner and Allard's (12) and Shirley's (41) studies with several species, and in Hurd-Karrer and Dickson's (20) studies with wheat.

The Mazda C tungsten lamp was compared with summer sunlight in a test with Marquis spring wheat. This variety was grown with a daily photoperiod of 15 hours outdoors during midsummer. One series received direct sunlight alone and another received 8 hours of direct sunlight supplemented by 7 hours of light from a Mazda C tungsten lamp delivering 8 to 10 foot-candles at the soil line.

Plants receiving full sunlight produced 14.2 tillers per plant and headed in 50 days, whereas those receiving both sunlight and electric light produced only 7.3 tillers per plant and headed in 46 days.

It seems likely that a reduction in the light intensity during part of the day, as well as the spectrum characteristic of the Mazda C tungsten lamp, caused the rapid completion of the vegetative period and sexual reproduction in this test. This lamp is especially strong in the yellow, orange, red, and infrared (7). Yellow, orange, and red have been reported to favor rapid development in certain plants (34, 38).

The retarding influence of bright sunlight on plant growth has been observed by others (40, 41). In the writers' tests, reducing summer sunlight to 41.3 percent reduced the heading time in Marquis to 50 days as compared with 55 days in unobstructed sunlight. Reducing the light to 27.9 percent increased the heading time to 58 days, and no heading took place when the intensity was reduced to 11.5 percent. The stems elongated most slowly in unobstructed sunlight, but the heads were more numerous and better filled than in the shaded series. Temperatures were equalized by means of fans.

COMPARISONS BETWEEN TUNGSTEN LAMP AND COOPER-HEWITT WORK LAMP

Tests were carried out for the purpose of comparing clear Mazda C tungsten lamps with a Cooper-Hewitt mercury-arc lamp having a lead-glass tube 50 inches long, as sources of light for lengthening the autumn and winter days.

The Mazda C lamp, as stated above, is especially strong in the infrared, red, and yellow, whereas the Cooper-Hewitt lamp emits yellow, green, blue, and violet, the red and orange being absent. The foot-candle intensities of the lamps were determined by means of the Macbeth illuminometer. Special screens were used in the illuminometer to procure this information for the Cooper-Hewitt lamp. Of the two factors (0.2 and 0.5) recommended for computing the foot-candle intensity of the Cooper-Hewitt lamp, the former was used. Two tungsten bulbs were used in white reflectors in order to obtain a uniformity of light distribution approaching that of the Cooper-Hewitt lamp. The plants received 8 hours sunlight and 8 hours artificial light daily. Temperatures ranged from 68° to 77° F.

Marquis spring wheat and Harvest Queen winter wheat served as test plants. Both chilled and unchilled seeds of Harvest Queen were used.



FIGURE 5.—*A* and *C*, Marquis spring wheat; *B* and *D*, Harvest Queen winter wheat from chilled seeds. *A* and *B*, grown with 8 hours of daylight and 8 hours of light from the Cooper-Hewitt mercury-arc lead-glass-tube lamp daily; *C* and *D*, grown with 8 hours of daylight and 8 hours of light from the Mazda C tungsten lamp daily.

It will be noted in table 4 and figure 5 that the tungsten source had a greater accelerating influence on the time of heading than did the Cooper-Hewitt lamp, but more tillers were produced under the Cooper-Hewitt lamp. Seed yields were not obtained, as mice destroyed some of the heads, but observations indicated that more heads and more seeds per head were produced in the plants grown under the Cooper-Hewitt lamp.

Mason (29), working with the date palm, observed that the Mazda lamp favored the pushing of the leaves from the growth center, whereas the Cooper-Hewitt lamp had an inhibiting influence.

TABLE 4.—*Period from planting to heading as influenced by the Mazda C tungsten lamp and the Cooper-Hewitt mercury-arc lamp with lead-glass tube, when used to supplement sunlight*¹

[Planted Sept. 19, 1930]

Variety and treatment of germinated seed	Mazda C tungsten lamp (44.7 foot-candles)	Cooper-Hewitt lamp (99.7 foot-candles)
Marquis:	Days	Days
Seed not chilled.....	50	81
Harvest Queen:		
Seed chilled for 65 days.....	50	85
Seed not chilled.....	(?)	(?)

¹ Lamps operated 8 hours before sunrise, followed by sunlight alone for 8 hours. Temperatures ranged from 68° to 77° F.

² There was no sign of stem elongation at the end of 90 days.

Seed yields were obtained in another test, however. Marquis spring wheat and five varieties of winter wheat from chilled seedlings were grown in a greenhouse near 70° to 75° F. during late autumn and winter. For the first 36 days the natural photoperiod of about 11 hours was maintained. After this period uninterrupted light was provided. In one-half of the series, daylight was supplemented with artificial light from the Cooper-Hewitt work lamp; in the other half, Mazda C tungsten lamps were the supplementary source. The lamps were turned on at sunset and off at sunrise. The light intensities in both tests were maintained as nearly as possible at 60 foot-candles at a point midway between the soil and the top of the plant.

In table 5 it will be observed that when the Cooper-Hewitt lamp was used the seed yield was increased in all varieties except White Winter. The White Winter plants were less uniform in their growth than the other varieties, and this may account for their response. There were not many plants in the winter varieties, but there was a reasonably large number in Marquis.

TABLE 5.—*Seed production as influenced by the Mazda C tungsten lamp and by the Cooper-Hewitt mercury-vapor lamp with lead-glass tube*¹

[Seeds were planted Oct. 13, 1931]

Variety	Plants under—		Heads produced under—		Seeds per head produced under—	
	Mazda C tungsten lamp	Cooper-Hewitt lamp	Mazda C tungsten lamp	Cooper-Hewitt lamp	Mazda C tungsten lamp	Cooper-Hewitt lamp
	Number	Number	Number	Number	Number	Number
Harvest Queen.....	2	2	10	7	10.9	18.8
Crimean.....	2	2	10	8	9.6	16.5
Minhardi.....	2	2	8	7	15.7	19.4
White Winter.....	2	2	11	10	13.7	10.9
Sol.....	2	2	7	10	5.29	13.5
Marquis.....	20	20	28	29	1.35	2.93

¹ During the first 30 days the natural photoperiod obtained, after which uninterrupted illumination was provided, the augmenting sources operating from sunset to sunrise.

The following winter another test was carried out with F_1 plants from winter-wheat crosses. The germinated chilled seeds were planted in the greenhouse. The temperatures were about 70° to 75° F. and the daily photoperiod was about 16 hours. Mazda C tungsten lamps were used after sunset. This photoperiod was maintained until the flag leaves appeared, when the series was divided into two groups of 16 plants each. Each group contained exactly the same progenies, and the plants were selected for uniformity of development. One group was placed under tungsten lamps and the other under the Cooper-Hewitt lamp combined with tungsten lamps. The light intensities in this test were maintained as nearly as possible at 120 foot-candles at a point midway between the soil and the top of the plant. The plants received full daylight. The lamps were turned on at sunset and off at sunrise.

Seed counts were made on the primary head of each plant. The 16 primary heads under the Cooper-Hewitt and Mazda C lamp combination produced 314 seeds, and the 16 primary heads under the Mazda C lamps produced only 216 seeds. The number of seeds per head ranged from 16 to 22 under the combined lamps and from 10 to 17 under the Mazda C lamps.

Very poor seed sets have been obtained when wheat plants begin to head in December and early January. Even when the day was lengthened artificially, the heads were frequently poorly developed and fertilization usually was not normal. Tests carried out in reduced sunlight in midsummer give similar results, thus indicating that the low intensity of daylight may be the chief cause of the poor heads in midwinter. All evidence seems to indicate that the success of a long day in which artificial light is used depends to a large extent on the amount of sunlight available.

CONCLUSIONS AND SUMMARY

Sexual reproduction in the spring wheats and in the winter wheats is not dependent on a critical temperature or a critical photoperiod, as this process occurs over very wide ranges of these factors. However, the time when sexual reproduction occurs is greatly influenced by the temperature and the photoperiod.

The proper classification of wheat varieties with respect to their temperature and photoperiodic characteristics for the earliest sexual reproduction is dependent on methods that make it possible to determine the optimum conditions of temperature and the photoperiod for the rapid completion of each growth phase, from the beginning of growth through sexual reproduction.

Spring wheats such as Marquis and earlier varieties complete their life cycle quickly when given a long day and temperatures at 70° F. or above throughout the life cycle, and therefore are considered to be typical long-day high-temperature plants. On the other hand, Harvest Queen, Turkey, and other varieties of winter wheat complete their life cycle most rapidly when given a short day and low temperatures during the early stages of growth and a long day and high temperatures during the later stages of development.

In the light of these relationships the writers conclude that winter wheats are not typical long-day plants but are what may be termed short-day→long-day plants and low-temperature→high-temperature

plants. This method of expression indicates that the temperature and the length of the photoperiod must increase with the development of the plant in order to induce early sexual reproduction.

Temperatures and photoperiods favoring earliness in the winter and spring wheats favor the formation of a reduced number of internodes and leaves by each tiller. The formation of the stem internodes and leaves stops and the major elongation of the stem begins at about the time that floral differentiation becomes evident.

When the Mazda C tungsten lamp was used to lengthen the day, Marquis headed sooner than when the same photoperiod consisted of sunlight alone. Heading was earlier under the Mazda lamp than under the Cooper-Hewitt lamp when these light sources were used to lengthen the winter day.

In 5 of the 6 varieties tested the number of seeds was greater when the winter day was lengthened by means of the Cooper-Hewitt lamp alone, or when in combination with the Mazda lamp, than when the Mazda lamps were used alone for supplementing daylight. It appears that a combination of these lamps may be better for certain studies than the Mazda lamp alone for lengthening the natural day. The Mazda lamp stimulated earliness at the sacrifice of vegetation and seed yield, and the Cooper-Hewitt lamp apparently offset this by increasing vegetation and the yield of seed.

Earliness and other characteristics of the wheat plant are influenced by the intensity of the light, and while this influence is less than that of the daily photoperiod so far as concerns earliness of sexual reproduction, it is sufficient to warrant attention in experimental work.

LITERATURE CITED

- (1) ANONYMOUS.
1837. A SUGGESTION FOR THE COMING YEAR. *Cultivator* 4 (4): 64.
- (2) ADAMS, J.
1924. DOES LIGHT DETERMINE THE DATE OF HEADING OUT IN WINTER WHEAT AND WINTER RYE? *Amer. Jour. Bot.* 11: 535-539.
- (3) ————
1925. SOME FURTHER EXPERIMENTS ON THE RELATION OF LIGHT TO GROWTH. *Amer. Jour. Bot.* 12: 398-412.
- (4) ALLEN, R. L.
1850. AMERICAN FARM BOOK; OR COMPEND OF AMERICAN AGRICULTURE. 325 pp. New York.
- (5) BAYLES, B. B., and MARTIN, J. F.
1931. GROWTH HABIT AND YIELD IN WHEAT AS INFLUENCED BY TIME OF SEEDING. *Jour. Agr. Research* 42: 483-500, illus.
- (6) BORODIN, D.
1934. YAROVIZATION FORMULAS FOR WINTER OATS. 16 pp., illus. New York.
- (7) COBLENTZ, W. W., and STAIR, R.
1929. DATA ON ULTRA-VIOLET SOLAR RADIATION AND SOLARIZATION OF WINDOW MATERIAL. U. S. Dept. Com., Bur. Standards Jour. Research 3: 629-689, illus.
- (8) DOROSHENKO, A. V.
1927. PHOTOPERIODISM OF SOME CULTIVATED FORMS IN CONNECTION WITH THEIR ORIGIN. *Trudy Prikl. Bot. i Selek.* (Bull. Appl. Bot. and Plant Breeding) 17 (1): [167]-220, illus. [In Russian. English summary, pp. 218-219.]
- (9) ENOMOTO, N.
1929. ON THE PHYSIOLOGICAL DIFFERENCE BETWEEN THE SPRING AND WINTER TYPES IN WHEAT AND BARLEY. *Jour. Imp. Agr. Expt. Sta. Tokyo* 1: 107-138, illus. [In Japanese. English résumé, pp. 136-138.]

- (10) FORSTER, H. C., TINCKER, M. A. H., VASEY, A. J., and WADHAM, S. M.
1932. EXPERIMENTS IN ENGLAND, WALES, AND AUSTRALIA ON THE EFFECT OF LENGTH OF DAY ON VARIOUS CULTIVATED VARIETIES OF WHEAT. *Ann. Appl. Biol.* 19: 378-412, illus.
- (11) FRUWIRTH, C.
1918. DIE UMZÜCHTUNG VON WINTERGETREIDE IN SOMMERGETREIDE. *Ztschr. Pflanzenzücht.* 6: 1-46.
- (12) GARNER, W. W., and ALLARD, H. A.
1920. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. *Jour. Agr. Research* 18: 553-606, illus.
- (13) ——— and ALLARD, H. A.
1923. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. *Jour. Agr. Research* 23: 871-920, illus.
- (14) GASSNER, G.
1910. BEOBSACHTUNGEN UND VERSUCHE ÜBER DEN ANBAU UND DIE ENTWICKLUNG VON GETREIDE-PFLANZEN IM SUBTROPISCHEN KLIMA. *Jahresber. Ver. Angew. Bot.* 8: 95-163, illus.
- (15) ———
1918. BEITRÄGE ZUR PHYSIOLOGISCHEN CHARAKTERISTIK SOMMER UND WINTER-ANNUELLER GEWÄCHSE, INSBESONDERE DER GETREIDE-PFLANZEN. *Ztschr. Bot.* 10: [417]-480, illus.
- (16) HELLRIEGAL, H.
1883. BEITRÄGE ZU DEN NATURWISSENSCHAFTLICHEN GRUNDLAGEN DES ACKERBAUS. Braunschweig. *Landw. Ztg.* 1883: 435.
- (17) HURD-KARRER, A. M.
1929. RELATION OF LEAF ACIDITY TO VIGOR IN WHEAT GROWN AT DIFFERENT TEMPERATURES. *Jour. Agr. Research* 39: 341-350, illus.
- (18) ———
1930. THE FORMATIVE EFFECT OF DAY LENGTH ON WHEAT SEEDLINGS. *Jour. Md. Acad. Sci.* 1: 115-126, illus.
- (19) ———
1933. COMPARATIVE RESPONSES OF A SPRING AND A WINTER WHEAT TO DAY LENGTH AND TEMPERATURE. *Jour. Agr. Research* 46: 867-888, illus.
- (20) ——— and DICKSON, A. D.
1934. CARBOHYDRATE AND NITROGEN RELATIONS IN WHEAT PLANTS WITH REFERENCE TO TYPE OF GROWTH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS. *Plant Physiol.* 9: 533-565, illus.
- (21) HUTCHESON, T. B., and QUANTZ, K. E.
1917. THE EFFECT OF GREENHOUSE TEMPERATURES ON THE GROWTH OF SMALL GRAINS. *Jour. Amer. Soc. Agron.* 9: 17-21, illus.
- (22) KLAGES, K. H.
1926. METRICAL ATTRIBUTES AND THE PHYSIOLOGY OF HARDY VARIETIES OF WINTER WHEAT. *Jour. Amer. Soc. Agron.* 18: 529-566, illus.
- (23) KLIPPART, J. H.
1858. AN ESSAY ON THE ORIGIN, GROWTH, DISEASES, VARIETIES, ETC., OF THE WHEAT PLANT. *Ohio State Bd. Agr. Ann. Rept.* (1857) 12: 562-816.
- (24) LYSENKO, T.
1928. A STUDY OF THE EFFECT OF THE THERMIC FACTOR UPON THE DURATION OF THE DEVELOPMENTAL STAGES OF PLANTS. *Azerbaijan Plant Breeding Sta. Bull.* 3, 169 pp. [In Russian. English summary.]
- (25) MCKINNEY, H. H., and SANDO, W. J.
1930. THE BEHAVIOR OF WINTER WHEAT IN ARTIFICIAL ENVIRONMENTS. *Science* (n. s.) 71: 668-670.
- (26) ——— and SANDO, W. J.
1933. RUSSIAN METHODS FOR ACCELERATING SEXUAL REPRODUCTION IN WHEAT. *Jour. Heredity* 24: 165-166.
- (27) ——— and SANDO, W. J.
1933. EARLINESS AND SEASONAL GROWTH HABIT IN WHEAT. *Jour. Heredity* 24: 169-179, illus.

- (28) ——— SANDO, W. J., SWANSON, A. F., HUBBARD, V. C., SMITH, G. S., SUNESON, C. A., and SUTHERLAND, J. L.
1934. FIELD EXPERIMENTS WITH VERNALIZED WHEAT. U. S. Dept. Agr. Circ. 325, 8 pp.
- (29) MASON, S. C.
1925. THE INHIBITIVE EFFECT OF DIRECT SUNLIGHT ON THE GROWTH OF THE DATE PALM. Jour. Agr. Research 31: 455-469, illus.
- (30) MAXIMOV, N. A.
1929. EXPERIMENTELLE ÄNDERUNGEN DER LÄNGE DER VEGETATIONS-PERIODE BEI DEN PFLANZEN. Biol. Zentbl. 49: 513-543, illus.
- (31) ———
1930. PHYSIOLOGICAL CONTROL OF LENGTH OF THE VEGETATIVE PERIOD. 5th Internatl. Cong. Bot., Cambridge, Abs. Commun., pp. 275-276.
- (32) ———
1934. THE THEORETICAL SIGNIFICANCE OF VERNALIZATION. Imp. Bur. Plant Genetics, Herbage Pub. Ser. Bull. 16, 14 pp.
- (33) ——— and POJARKOVA, A. I.
1925. ÜBER DIE PHYSIOLOGISCHE NATUR DER UNTERSCHIEDE ZWISCHEN SOMMER- UND WINTERGETREIDE. Jahrb. Wiss. Bot. 64: [702]-730, illus.
- (34) PALLADIN, V. I.
1918. PLANT PHYSIOLOGY. English translation ... by B. E. Livingston. 320 pp., illus. Philadelphia.
- (35) PAPADAKIS, J.
1931. A STUDY OF THE EFFECT OF TEMPERATURE CONDITIONS DURING EARLY GROWTH UPON RELATIVE EARLINESS AND EARING OF SPRING WHEATS. COLD AS POSITIVE FACTOR OF WHEAT YIELD. Assoc. Internatl. Select. Plant Bull. 4: 98-102. [German and French pp. 102-105.]
- (36) PAPADAKIS, J. S.
1933. NON SEULEMENT LES VARIÉTÉS DE BLÉ D'HIVER MAIS AUSSI CELLES DE PRINTEMPS ONT BESOIN DE FROID POUR ÉPIER.—LA PRÉCOCITÉ RELATIVE DES VARIÉTÉS DÉPEND DES CONDITIONS DE TEMPÉRATURE DU PREMIER DÉVELOPPEMENT.—LE FROID COMME FACTEUR POSITIF DU RENDEMENT. Ann. Gembloux 39: 79-85.
- (37) POJARKOVA, A. J.
1927. TEMPERATURBEDINGUNGEN DER KEIMUNG ALS BESTIMMENDER FAKTOR FÜR ÄHRENBILDUNG BEIM WINTERGETREIDE. Ber. Deut. Bot. Gesell. 45: 627-637.
- (38) POPP, H. W.
1926. A PHYSIOLOGICAL STUDY OF THE EFFECT OF LIGHT OF VARIOUS RANGES OF WAVE LENGTH ON THE GROWTH OF PLANTS. Amer. Jour. Bot. 13: 706-736, illus.
- (39) RASUMOV, V. J.
1930. ÜBER DIE PHOTOPERIODISCHE NACHWIRKUNG IM ZUSAMMENHANG MIT DER WIRKUNG VERSCHIEDENER AUSSAATTERMEINE AUF DIE PFLANZEN. Planta, Arch. Wiss. Bot. 10: [345]-373, illus.
- (40) SHANTZ, H. L.
1913. THE EFFECTS OF ARTIFICIAL SHADING ON PLANT GROWTH IN LOUISIANA. U. S. Dept. Agr., Bur. Plant Indus. Bull. 279, 31 pp., illus.
- (41) SHIRLEY, H. L.
1929. THE INFLUENCE OF LIGHT INTENSITY AND LIGHT QUALITY UPON THE GROWTH OF PLANTS. Amer. Jour. Bot. 16: 354-390, illus.
- (42) WACAR, B. A.
1925. ZUR FRAGE ÜBER D. EINFLUSS TEMPERATUR AUF DIE BILDUNG DER ÄHREN BEI WINTERROGGEN UND WINTERWEIZEN. Jour. Landw. Wiss. Moskau 2: [776]-785, illus. [In Russian. German summary, p. 785.]
- (43) WANSEER, H. M.
1922. PHOTOPERIODISM OF WHEAT: A DETERMINING FACTOR IN ACCLIMATIZATION. Science (n. s.) 56: 313-315.
- (44) WHYTE, R. O., and HUDSON, O. S.
1933. VERNALIZATION OR LYSENKO'S METHOD FOR THE PRE-TREATMENT OF SEED. Imp. Bur. Plant Genetics (Herb. Plants) Bull. 9, 27 pp., illus.

THE ANATOMY AND HISTOLOGY OF THE TRANSITION REGION OF *TRAGOPOGON PORRIFOLIUS*¹

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INTRODUCTION

Salsify (*Tragopogon porrifolius* L.) is a biennial plant belonging to one of the most specialized groups of the Compositae. During the first year of growth it produces a fleshy primary root, a fleshy hypocotyl, and a very short stem. In its second year a flower stalk is developed from the stem which extends to a height of 2 to 3 feet and bears disks of purple flowers. The seedling has linear, lanceolate, sessile cotyledons, which carry on photosynthesis. The fruit (commonly called the seed) is a linear achene about 1 cm in length.

REVIEW OF LITERATURE

A search of the literature failed to reveal any reports dealing with the anatomy of the transition region of salsify. Van Tieghem (6)² in 1870 described very briefly the transition in a few plants of the Compositae, and Gérard (3) in 1881 reported the results of studies on the seedling anatomy of two members of the Compositae. One of these, *Carthamus tinctorius*, is very similar in its type of transition to *Tragopogon porrifolius* in most details mentioned relative to the root and lower hypocotyl. Vuillemin (8) reported studies with the stems of some of the composites and included very brief notes on the transition phenomena. Dangeard (2) made very general observations on types of transition and based the results of his final classification on the number of primary xylem groups in the root. Van Tieghem (7, p. 782) defined three general types of transition from root to stem. Compton (1) described several types of syncotily in the Compositae. His study was made from an evolutionary viewpoint. Hill and De Fraine (4) investigated seedling structure from two general aspects—phylogeny and physiology. They state (4, p. 262):

The existence of an intermediate type between diarchy and tetrarchy has been demonstrated in certain plants, e. g. *Liriodendron tulipifera*, *Clematis Hendersonii*, and some of the Composites; in these, lateral bundles of the cotyledons enter the hypocotyl and attempt to form the intercotyledonary poles of a tetrarch root.

Lee (5) reported very briefly his work on the seedling anatomy of a large number of plants belonging to the Compositae. Of these only *Heliopsis laevis* (seedling A), *Centaurea macrocephala*, and *Tragopogon dubius* seem at all similar to salsify. However, in Lee's article, as well as in most of the others cited, very little detail is given, and one cannot be certain as to the amount of similarity in structure. The present paper presents the results of a study of the anatomical and histological changes that take place in the transition region of salsify.

¹ Received for publication May 6, 1935; issued December 1935.

² Reference is made by number (italic) to Literature Cited, p. 654.

MATERIAL AND METHODS

The Mammoth Sandwich Island variety of salsify was used for this study. The plants were grown in the greenhouses of the Ohio State University during the summer and fall of 1934. Seedlings of various ages were used, but those 6 to 8 days old proved to be the best material for a detailed study of the root-stem transition. At this stage all the primary tissues were differentiated but no secondary thickening was present.

The specimens were fixed in formol acetic alcohol, dehydrated according to the usual method, run through chloroform, and embedded in paraffin. Serial transverse and longitudinal sections were cut 10μ to 15μ in thickness, mounted serially, and stained in most cases with safranin and fast green.

GROSS MORPHOLOGY OF YOUNG SEEDLING

The first indication of germination is the bursting of the seed coat and the hard outer covering (composed of carpel wall and receptacle) at the base of the achene, by the elongation of the hypocotyl and growth of the radicle. The elongation of the inverted V-shaped hypocotyl brings the cotyledons, which are still enclosed by the fruit coat, out of the ground. In about a day the hypocotyl straightens, and the plantlet assumes a vertical position. The walls of the achene and seed coat usually remain, partly enclosing the growing cotyledons for a day or two, then are shed completely. In the greenhouse at a temperature of 60° to 70° F. this entire process requires from 5 to 6 days. The cotyledons are parallel-veined and sessile. At their base they are undiverged, thus forming a tube surrounding the epicotyl, but are separate at almost a centimeter above the cotyledonary node. The epicotyl grows very slowly at first, and several days elapse before elongation takes place.

ANATOMY OF THE YOUNG PRIMARY ROOT

The primary xylem of the salsify root is diarch; no exceptions to this were found. As is usual in roots, the xylem and phloem are radially arranged in the protostele (fig. 1). Within about 7 days after the seed is planted the primary body is differentiated. There are 6 to 10 cells in each protoxylem group abaxially arranged with respect to the 10 to 15 central metaxylem elements. These vessels are spirally thickened both in root and hypocotyl. Several layers of parenchymatous cells, one of which will form the primary cambium, separate the primary phloem from the diarch xylem. The primary phloem is composed of sieve tubes, companion cells, and phloem parenchyma.

The pericycle is composed of one layer of rather irregularly shaped parenchymatous cells. It surrounds the primary phloem in a continuous sheath and abuts the protoxylem points. The cells are longer vertically than in their radial diameter. Lateral roots originate from the pericycle opposite the protoxylem points in all cases noted. The Casparian thickenings of the endodermis are fairly conspicuous, making this tissue readily recognizable. The cortex is composed of parenchymatous cells 10 to 12 layers in thickness. They are loosely arranged and irregular in shape. Intercellular

spaces are comparatively large in this tissue. The epidermis is one layer thick in the young root. The cells are very much greater in their vertical than in their radial dimension. The epidermis is the first tissue to differentiate out of the promeristem; followed closely by the cortex. No fibers are associated with the root or the hypocotyledonary bundles in the primary body.

THE TRANSITION REGION

The word "hypocotyl" as used in this paper refers to that morphological part of the plant between the cotyledonary node and the root. The hypocotyl may be easily located in many seedlings. Its

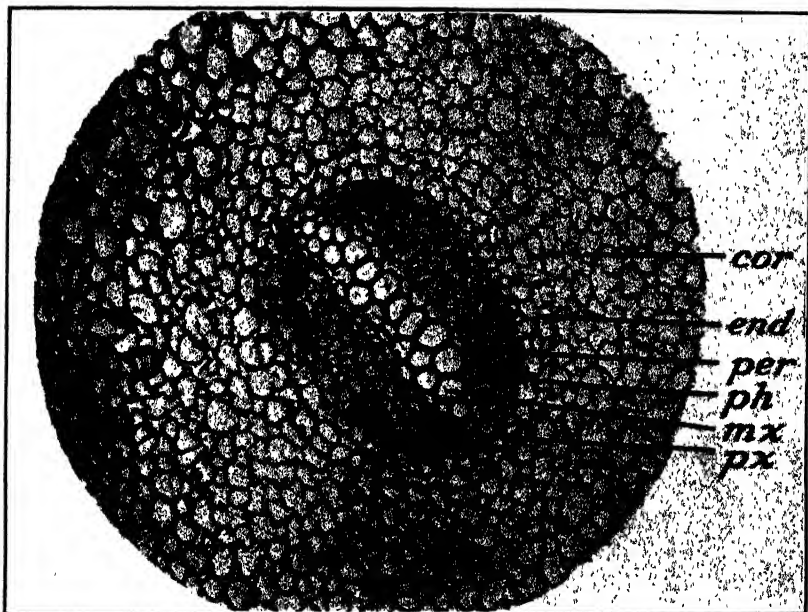


FIGURE 1.—Transverse section of young primary root; *px*, protoxylem; *mx*, metaxylem; *ph*, phloem, *per*, pericycle; *end*, endodermis; *cor*, cortex.

upper extremity is characterized by the divergence of the cotyledons. Its lower approximate terminus may usually be distinguished by the definite constriction which characterizes the beginning of the root structure. This is true in the case of the plant here described. The hypocotyl is not necessarily accompanied by anatomical changes throughout its entire length.

The term "transition region", as here used, refers only to that portion of the plant in which definite anatomical and histological changes take place in the reorientation of structures between root and stem. In many plants which possess a fleshy hypocotyl the transition region is located only in the upper portion of the hypocotyl. In the salsify the transition region is located entirely in the hypocotyl, although it includes only approximately the upper half of it, the lower portion being rootlike in anatomical structure.

The various changes occurring in the transition from root to stem structure of the primary body are given below, beginning with the

root and continuing through the hypocotyl to the base of the cotyledons where the transition is complete. The lower portion of the hypocotyl is diarch and rootlike in structure. Four primary xylem groups are present in the upper portion of the hypocotyl. The two primary xylem groups found in the root are continuous through

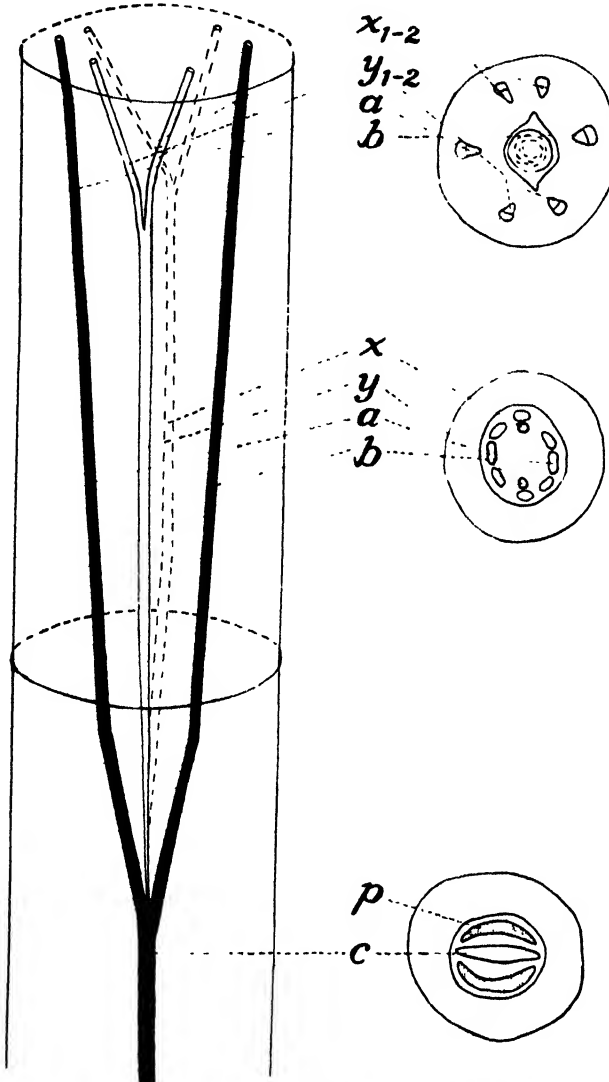


FIGURE 2.—Diagrammatic representation of vascular arrangement in transition region: c , Diarch xylem of lower region; p , primary phloem; a , b , cotyledonary bundles; x , y , lateral bundles; x_{1-2} , y_{1-2} , divergences of x and y respectively.

the whole length of the hypocotyl and diverge in their entirety into the cotyledons, making up, respectively, the midribs of each of the two sessile cotyledons. They are always on the same side as the cotyledons into which they diverge (fig. 2, a and b) and will hereafter be referred to as cotyledonary bundles.

Two bundles, in addition to the above-mentioned ones, arise in the hypocotyl. They are differentiated slightly later than the cotyledonary ones. In the upper portion of the hypocotyl these bundles are located laterally and at right angles with respect to the cotyledonary ones; hence they will hereafter be referred to as lateral bundles (fig. 2, *x* and *y*).

Some variation occurs in the origin of the lower portion of the lateral bundles in different individuals. In most instances the bundles are formed, in the lower transition region, from metaxylem which is continuous with that of the primary root (fig. 3). In some plants, however, the xylem is not continuous with the root, but originates at a higher level. In such cases pith cells separate the lower ends of the tracheae from those of the cotyledonary bundles and the lateral

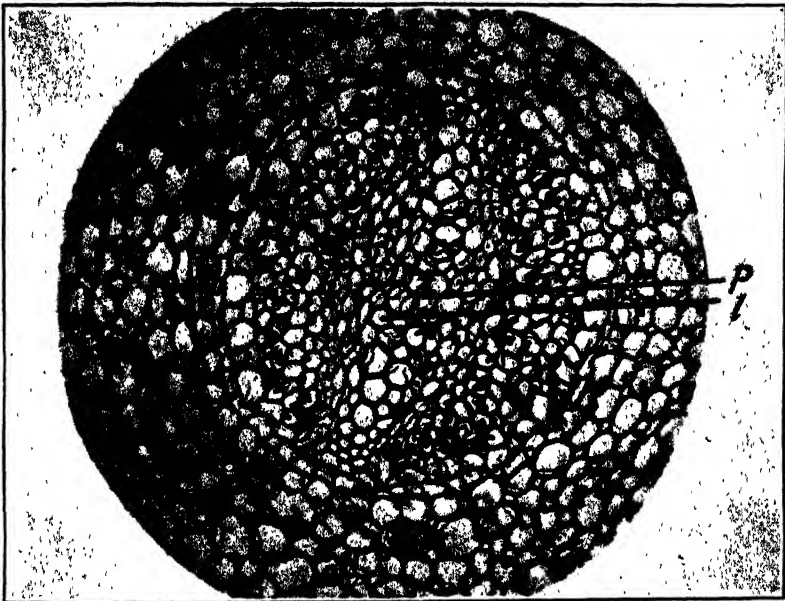


FIGURE 3—Section through lower transition region showing origin of lateral bundles from metaxylem: *l*, Xylem of lateral bundles; *p*, pith.

bundles are differentiated directly out of the pith tissue which separates the two cotyledonary bundles. When this occurs, the primary xylem of the lateral bundles has no direct vascular connection with that of the diarch root. The primary phloem of all the bundles is continuous, however. There are also intermediate stages between these two situations. The metaxylem of the lower hypocotyl is sometimes continuous with that of the lateral bundles in a few strands only, with many pith cells interspersed among them. In other instances there are only a few pith cells, in which case several vessels of the lateral bundles are continuous with those of the lower hypocotyl and root.

At a slightly higher level the primary xylem of each of the cotyledonary bundles is farther apart, and pith is laid down between these two groups and thus also between xylem of the lateral bundles differentiating between them (fig. 4). More metaxylem is then differen-

tiated in this central mass, and finally two distinct groups appear. This xylem, at the level here described, is neither exarch nor endarch but forms the primary xylem of two transition bundles (fig. 5). In fact, these lateral bundles are never exarch or radial during their ontogeny. They are in a transition stage at the distal region from the cotyledonary node, and endarch collateral bundles in the upper portion of the hypocotyl. At first considerable parenchyma occurs between the xylem and phloem, but the xylem is laid down nearer and nearer to the phloem, until definite collateral bundles are organized. At this level there is no change in the exarch arrangement of the xylem in the cotyledonary bundles and they are still radial with

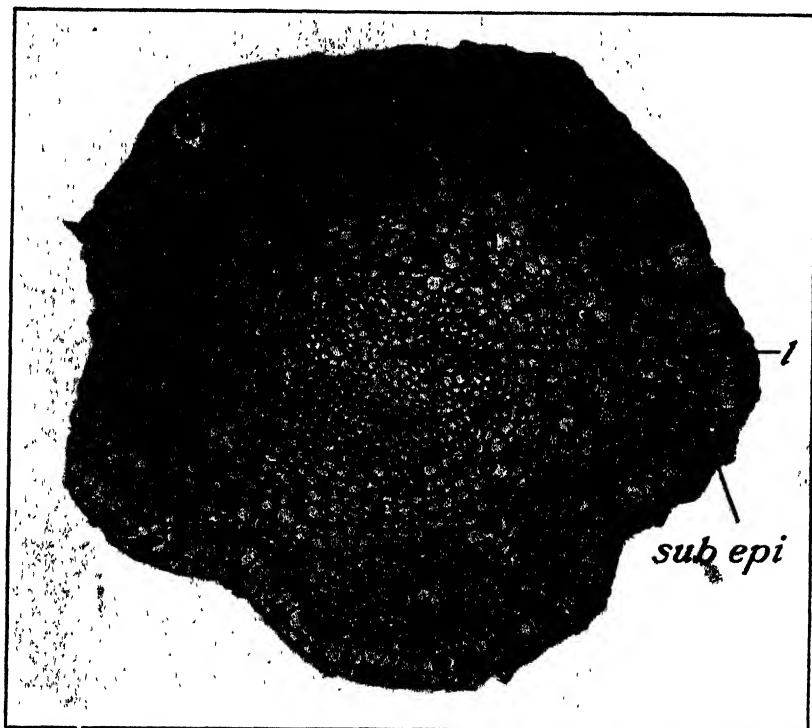


FIGURE 4.—Transverse section through lower transition zone. Note origin of lateral bundles (*l*) from metaxylem between cotyledonary bundles and also subepidermis (*sub epi*).

respect to the phloem. The pericycle is sometimes composed of two layers of cells opposite the protoxylem points at this position. Lateral roots originate from the pericycle opposite these points. The endodermis is structurally the same here as in the root. There are a few more layers of cortical cells, however. Two layers of epidermal cells begin to appear. Both layers are composed of large cells (fig. 5). The outer one, at its lower extremity, gives rise to numerous root hairs.

Farther up the hypocotyl the four bundles become more radially symmetrical, with a pith composed of loosely arranged parenchymatous cells. Within about 1 mm from the basal origin of the lateral bundles, all four of them are equally distributed at 90-degree intervals

from each other as one views them in transverse section. The lateral xylem groups are here collaterally arranged with respect to the two phloem groups. The phloem just outside of them is gradually reorientated until, at higher levels, it makes up the primary phloem of each of the lateral bundles. Here is found the first indication of transition of the primary cotyledonary bundles from exarch to endarch arrangement (fig. 7). The metaxylem is differentiated abaxially and on either side of the protoxylem. The protoxylem is then adaxially differentiated and thus through the center of the group of metaxylem cells. The lateral bundles are, at this level, still transition bundles. No appreciable change is evident in the position of the primary phloem.

At only a slightly higher level, and throughout most of the remaining upper hypocotyledonary region, the cotyledonary bundles are

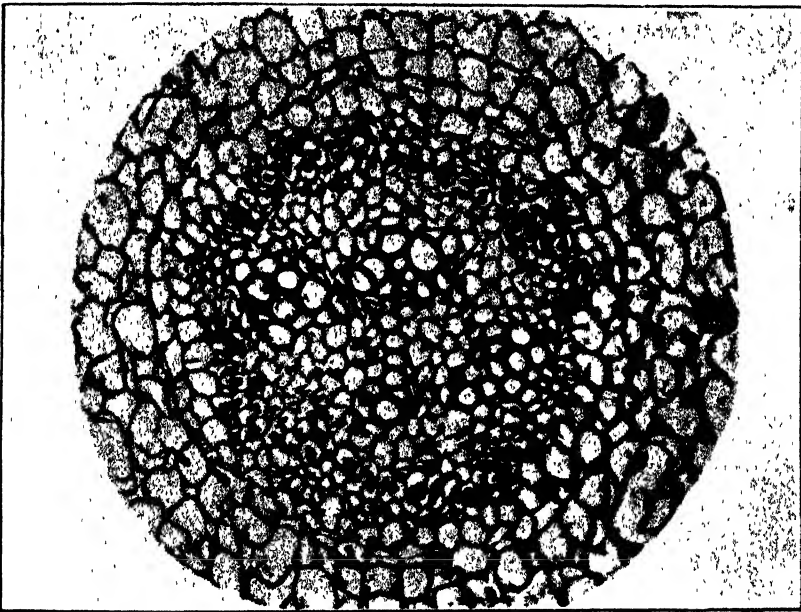


FIGURE 5.—Section through transition zone showing differentiation of two xylem groups between the cotyledonary bundles.

transition ones but are radial with respect to their primary xylem and phloem. At this level the xylem of the lateral bundles is more endarch than transitional and the bundles are nearly collateral. At a slightly higher level the cotyledonary bundles are transition radial ones and the two lateral bundles are perfectly endarch and collateral (figs. 6 and 7). Adaxial differentiation of the protoxylem has resulted in this endarch condition of the lateral bundles. In the cotyledonary bundles the abaxial differentiation of the metaxylem takes place laterally on both sides of the protoxylem. The pericycle at this level is two layers in thickness and the endodermis is still present but is irregular in shape.

At a position about 1 mm below the cotyledonary plate each phloem group associated radially with the cotyledonary bundles bifurcates,

forming two irregular groups. The two groups on either side and adjacent to the xylem of the cotyledonary bundles anastomose immediately outside of this xylem, and thus collateral bundles are formed. However, the xylem of these bundles is still in a transition stage. Small strands of the remaining groups of phloem, located between each cotyledonary and each lateral bundle, diverge into the cortex (fig. 8). A part of this phloem is continuous to a higher level, where it forms a ring of cells inside of the diverging primary vascular bundles. These phloem cells gradually disappear at higher levels

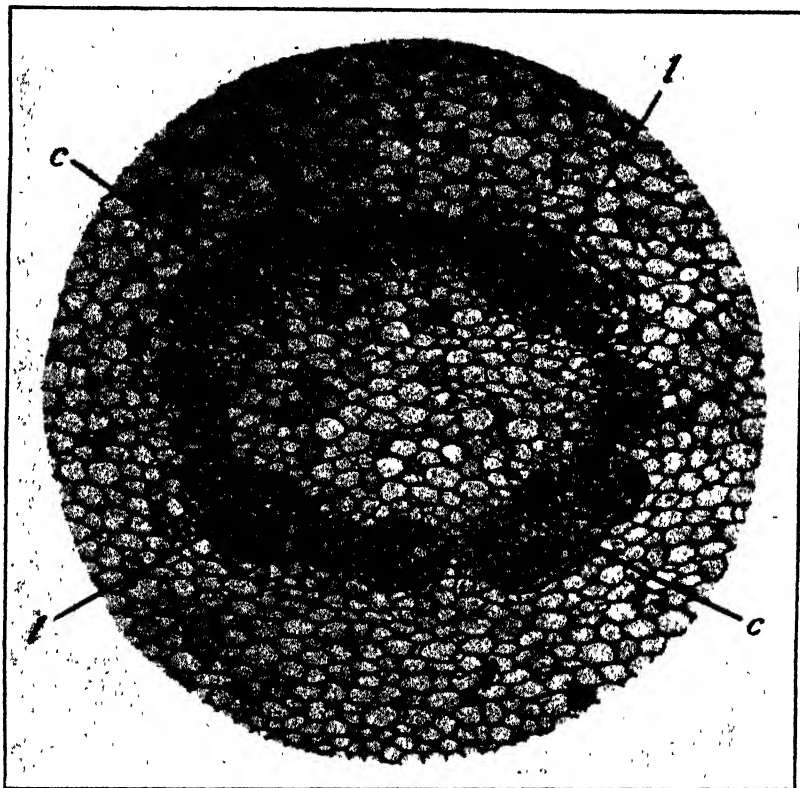


FIGURE 8.—Transverse section through upper hypocotyl. Note transition and radial arrangement of cotyledonary bundles (c) and endarch collateral position of lateral zones (l). $\times 100$.

until the base of the epicotyl is reached, which is composed of a mass of undifferentiated meristematic cells. The primary phloem of the root thus in part seems continuous with that of the stem. The primary xylem, however, is not continuous with that of the stem but diverges entirely into the cotyledons. Only the secondary xylem of the root is continuous with the xylem of the stem.

The xylem of the cotyledonary bundles is not endarch at the same level that the bundles are collateral but becomes so at a slightly higher level, and is entirely so at the position of the bundle divergence toward the cotyledons. At this same level each of the lateral bundles bifurcates, forming two closely associated bundles (fig. 9). These gradually are laid down farther apart until they finally form a lateral trace in

each of the two cotyledons (fig. 10). They always diverge and form the cotyledonary traces on the same side of the plant as that on which they arose and extend throughout their length in the hypocotyl. The cotyledonary bundles diverge at the same level and make up the midribs of the cotyledons.

Since salsify is definitely syncotyledonous, there is no sharp divergence of the cotyledonary traces, but they extend for some distance through the cotyledons in an almost vertical position.

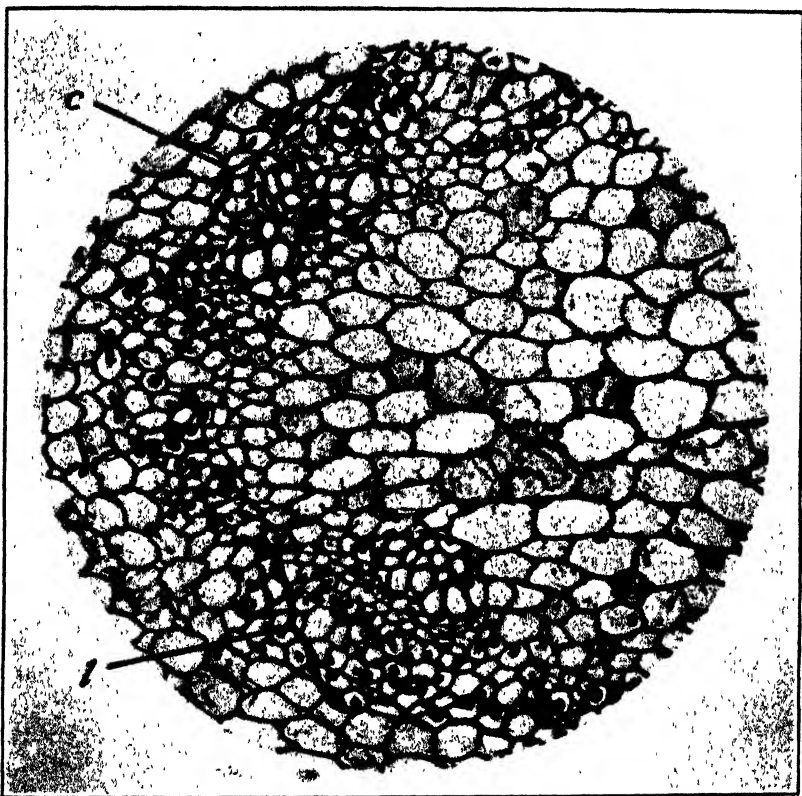


FIGURE 7.—Higher magnification of section shown in figure 6; note same points there suggested. $\times 250$

ADDITIONAL OBSERVATIONS

In connection with the foregoing studies, it was noted that no oil ducts are present in either the young primary or the mature salsify. The substance commonly spoken of as "milky" is found mainly in the phloem.

The edible portion of the mature salsify is composed largely of xylem. Most of this is xylem parenchyma. The phloem, pericycle, endodermis, and cortex are also present in the mature structure. In striking contrast to the situation in such plants as the radish, carrot, and beet, in which the cork cambium develops from the pericycle, in the salsify it develops from cortical tissue. The endodermis is readily distinguished in the mature structure by the prominent Casparian strips.

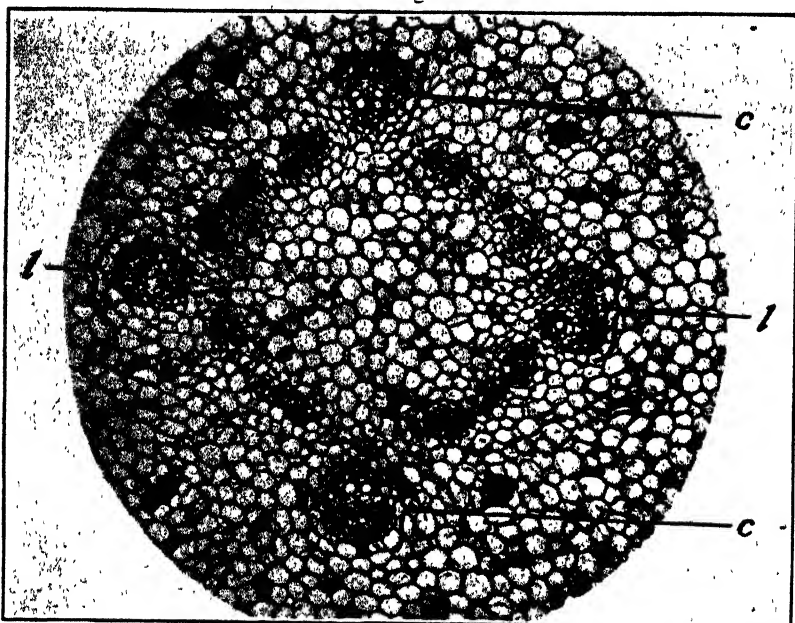


FIGURE 8.—Transverse section through upper portion of hypocotyl. Note primary phloem between the bundles and strands of it through the cortex: *l*, Lateral bundles; *c*, cotyledonary bundles.

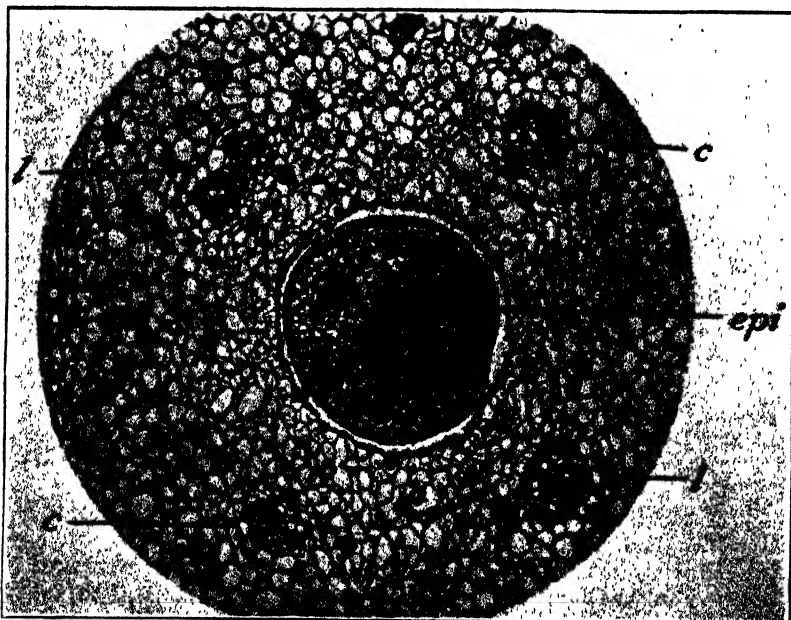


FIGURE 9.—Transverse section at base of epicotyl; lateral bundles slightly bifurcated and all bundles diverging into cotyledons: *l*, Lateral bundles; *c*, cotyledonary bundles; *epi*, base of epicotyl.

SUMMARY

The salsify seedling has linear, lanceolate, sessile cotyledons which are photosynthetic. The slowly developing epicotyl is enclosed in a cotyledonary tube to about 1 cm above the cotyledonary node.

The transition phenomena were studied from the root through the hypocotyl to the bases of the cotyledons where the transition is complete.

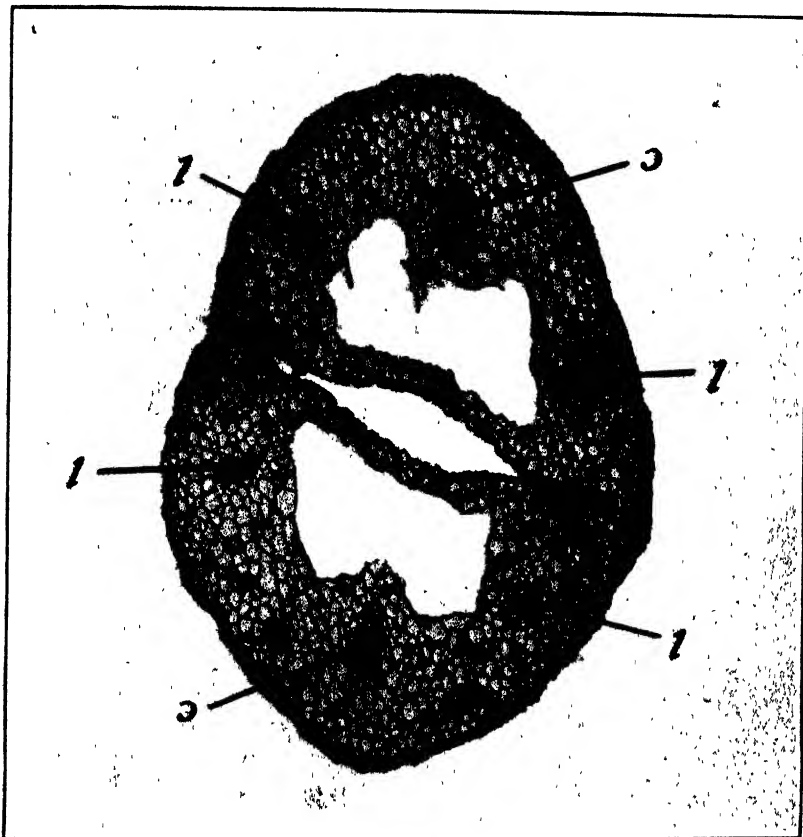


FIGURE 10.—Transverse section through cotyledons. Note lateral bundles become lateral traces of the cotyledons, while cotyledonary bundles become midribs of the cotyledons: *l*, Lateral bundles; *c*, cotyledonary bundles. The central cavity represents the cotyledonary tube; the larger cavities show the breaking down of the central parenchyma of the cotyledons.

The transition from root to stem structure occurs entirely in the hypocotyl.

The root is diarch and the primary xylem and phloem are radially arranged as is typical in the primary structure of roots.

The transition region is largely tetrarch; two primary bundles, in addition to the two continuous with those of the root, being differentiated in the lower portion of the transition zone from metaxylem.

Both the phloem and the xylem in the two primary bundles of the root are continuous through the hypocotyl and into the midribs of the cotyledons.

The two additional bundles arising in the basal portion of the transition region are located laterally and at right angles with respect to the cotyledonary bundles.

Each lateral bundle separates near the cotyledonary node and the two strands thus formed make up a lateral trace in each of the two cotyledons.

The transition from the exarch radial arrangement of the primary cotyledonary bundles to the endarch collateral arrangement extends through the upper one-half of the hypocotyl, hence almost to the cotyledonary node.

During this transition the metaxylem vessels are differentiated abaxially with respect to the protoxylem and laterally on either side of it.

The primary cotyledonary bundles are radially arranged with respect to the xylem and phloem throughout most of the transition zone, but are typical collateral bundles where they diverge into the cotyledons.

The lateral bundles in the hypocotyl are never exarch or radial, but are laid down as transition bundles in the lower transition region and extend thus throughout most of this zone. However, there are endarch collateral ones at a lower level than those of the cotyledonary bundles.

These four bundles of the primary body extend through most of the hypocotyl in a radially symmetrical pattern.

LITERATURE CITED

- (1) COMPTON, R. H.
1913. AN ANATOMICAL STUDY OF SYNCOTYLY AND SCHIZOCOTYLY. *Ann. Bot.* [London] 27: [793]-821, illus.
- (2) DANGEARD, P. A.
1889. RECHERCHES SUR LE MODE D'UNION DE LA TIGE ET DE LA RACINE CHEZ LES DICOTYLÉDONES. *Botaniste* 1: [75]-125, illus.
- (3) GÉRARD, R.
1881. RECHERCHES SUR LE PASSAGE DE LA RACINE A LA TIGE. *Ann. Sci. Nat., Bot.* (6) 11: [279]-430, illus.
- (4) HILL, T. G., and DE FRAINE, E.
1913. A CONSIDERATION OF THE FACTS RELATING TO THE STRUCTURE OF SEEDLINGS. *Ann. Bot.* [London] 27: [257]-272, illus.
- (5) LEE, E.
1914. OBSERVATIONS ON THE SEEDLING ANATOMY OF CERTAIN SYMPETALE. II. COMPOSITAE. *Ann. Bot.* [London] 28: [303]-329, illus.
- (6) VAN TIEGHEM, P.
1870-71. RECHERCHES SUR LA SYMÉTRIE DE STRUCTURE DES PLANTES VASCULAIRES. *Ann. Sci. Nat., Bot.* (5) 13: [5]-314, illus.
- (7) ———
1891. TRAITÉ DE BOTANIQUE. Éd. 2, rev. et augm., 1885 pp., illus. Paris.
- (8) VUILLEMIN, P.
1884. DE LA VALEUR DES CARACTÈRES ANATOMIQUES AU POINT DE VUE DE LA CLASSIFICATION DES VÉGÉTAUX. TIGES DES COMPOSÉES. 257 pp. Paris.

EFFECT OF INTERIOR TEMPERATURES OF BEEF MUSCLE UPON THE PRESS FLUID AND COOKING LOSSES¹

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INTRODUCTION

Quality and palatability of lean tissue of meat are largely dependent upon their physicochemical relationships. In examining the factors contributing to the edible qualities of meat, investigators have emphasized the importance of tenderness, juiciness, flavor, and aroma. Research dealing with these qualities has been effective in advancing scientific knowledge in meat cookery. Physicochemical investigation is necessary, however, to bring about a clear understanding of the changes that take place when meat is heated.

A method for studying the quality and quantity of press fluid or juiciness in different meat samples has been developed through the use of the pressometer (4).² The term "press fluid" is used to designate the fluid, consisting of moisture plus the soluble material plus the colloidal fraction, expressed from muscle by the use of the pressometer. This paper deals with a study of the quantity and composition of press fluid from beef muscle heated to different temperatures to determine their effect on the palatability of meat with respect to juiciness, a very important factor in meat quality.

REVIEW OF LITERATURE

The factors that affect the cooking losses of meat when roasted have been the subject of many investigations. Early work by Grindley and Mojonner (9) dealt with the losses of meat cooked in water and by dry heat. Their results show that dry heat caused losses of from 0.25 to 4.55 percent of the nitrogenous matter and 2.47 to 27.18 percent of the fat. Cline and Godfrey (5) concluded that loss in weight varied directly with increase in temperature. Grindley and Emmett (8) studied the juice and fiber of meat cooked in different ways. The juice was removed from raw ground meat by pressure, the yield being approximately 30 cc per 100 g. On heating, the red juice changed to brown at 52° C. These investigators found that the juice had the distinctive flavor of meat, while the fibers had little or no flavor though prepared in different ways. The flavor of the juice was more pronounced in the liquid portion than in the coagulated precipitate. Cline, Trowbridge, Foster, and Fry (6) found, from judges' grading, that increased shrinkage was accompanied by a decrease in tenderness, juiciness, and flavor of lean meat and that the loss of flavor might be attributed to loss of juices.

¹ Received for publication Apr. 8, 1935; issued December, 1935. Scientific Journal Series, Paper No. 1343, Minnesota Agricultural Experiment Station.

² Reference is made by number (italic) to Literature Cited, p. 661.

Early investigation of pure muscle fluid—fluid removed from muscle not in contact with either water or salt—was carried on by Botazzi (3) in 1912. He obtained fluid from raw ox muscle that had been stored on ice and then chopped and ground with diatom powder. By means of a hydraulic press, using pressures varying from 50 to 350 atmospheres, he expressed the fluid in quantities sufficient for the determination of its physical and chemical properties. Because of the general inaccessibility of Botazzi's work, certain of his data are shown in table 1.

TABLE 1.—Yield of press fluid, at 350 atmospheres pressure, from raw ox muscle, and dry residue, protein, and ash content of the fluid¹

Muscle	Weight of muscle	Weight of fluid	Yield	Dry residue	Total protein	Ash	Residue protein and ash
	<i>Kilograms</i>	<i>Kilograms</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Striated.....	0.938	0.529	63	8.914	91.086	1.739	2.654
Smooth.....	.829	.339	40	7.43	92.57	.85	2.93

¹ Botazzi (3).

Other analyses of fluid from ox muscle are given by Wiley (14), Leach (11), states that meat juice is the fluid portion of the muscle fibers. The standard for true meat juice adopted by the Association of Official Agricultural Chemists (1) is:

The solids contain not more than 15 percent of ash, not more than 2.5 percent NaCl, not more than 4 nor less than 2 percent phosphoric acid, and not less than 12 percent of nitrogen. The nitrogenous bodies contain not less than 35 percent of coagulated proteins and not more than 40 percent of the meat basis.

Halliday, Noble, and Klaas (10), using a pressure of 3,800 pounds per square inch for expressing juice, found that rib roasts heated to 61° C. yielded a larger quantity of juice than rib roasts heated to 75°. The quantities of juice ranged about 15 cc per 100g. The juice from roasts heated to 61° was richer in solid and total nitrogen than that from roasts heated to 75°.

Bigelow and Cook (2) showed that a larger yield of juice could be obtained from meat heated to 60° C. than from raw muscle.

The methods and technique for press-fluid analyses, have been described by Child and Baldelli (4), who used 250 pounds pressure on the pressometer for 10 minutes. A study of sampling by Zohner³ proved that duplicate samples from the same slice could be used for analysis, since the difference between them was not significant.

MATERIAL AND METHODS

The semitendinosus or eye muscle from the round of beef contains all of the structural components of striated muscle in a convenient compact unit. The entire raw cut weighs from 1 to 1.5 kg and measures from 25 to 30 cm in length and from 6 to 8 cm in diameter, being approximately cylindrical in shape and tapering abruptly at either end. The end pieces were not used for cooking. The muscle was removed from the round by cutting along the seam between the eye

³ ZOHNER, E. T. A STUDY IN SAMPLING FOR PRESS FLUID INVESTIGATION. 1934. (Unpublished material, Minnesota Agricultural Experiment Station.)

and the top round and separating the muscle from the bottom round. The beef obtained from a large packing house was of choice quality and was ripened 14 days. An electric oven with an automatic control was used for cooking, and a pressometer was used for removing the fluid from the muscle.

PREPARING AND ROASTING THE MEAT FOR SAMPLING

Two roasts from the same muscle were prepared according to the method given in quality tests by the cooking committee of the cooperative meat investigation committee.⁴ All exterior fat was carefully removed, leaving a continuous covering of connective tissue surrounding the muscle. Preliminary work showed that the meat was more uniformly cooked when prepared in this manner. The anterior portion was used for the tests at 58° C., and the posterior section for those at 75°. The length of the roast was determined by its diameter to insure even heat penetration to the center from all sides. The meat was cooked until the thermometer registered 58° for the rare and 75° for the well-done roasts. The former temperature was chosen because it is used for rare roasts by the cooking committee of the cooperative meat investigation committee.⁴ The higher temperature was decided upon after a series of preliminary roasting experiments had been performed to determine the temperature at which the standard of the center slice of the roast would conform to that for well-done beef given by Sprague and Grindley (13), who describe the interior of meat cooked to 75° to be brownish gray in color, with a scant amount of colorless or slightly yellow juice.

Total losses, the sum of the evaporation and dripping losses, were obtained.

SAMPLING FOR PRESS-FLUID DETERMINATION

After cooking, the roasts were cooled to 40° C. They were then halved, and a slice 1.25 cm in thickness was removed from the center. On either side of the thermometer duplicate samples were taken by means of a round cylindrical borer 1.25 cm in diameter. The weighed sample was wrapped in an unsized, shrunk, weighed filter cloth and put in the tray of the pressometer under a pressure of 250 pounds for 10 minutes and weighed again. The weight of the press fluid was found by subtracting the weight of the pressed sample from the weight of the unpressed sample.

To obtain the quantity of press fluid per gram of dry matter, the expressed sample after preliminary evaporation in a drying oven was brought to constant weight at a temperature of 80° C. ± 2 under high vacuum. The ratio of press fluid to dry matter was calculated by dividing the weight of the press fluid by the weight of the dried sample.

OBTAINING PRESS FLUID FOR CHEMICAL ANALYSIS

For chemical analysis of the press fluid, the slice which had been sampled for press-fluid determination was used, the outer layer 1.25 cm in thickness being removed and the remainder cut into 1.25-cm cubes.

⁴ ALEXANDER, L. M., CLARK, N. G., and HOWE, P. E. METHODS OF COOKING AND TESTING MEAT FOR PALATABILITY. Supplement to National Project Cooperative Meat Investigations. U. S. Dept. Agr., Bur. Home Econ. and Bur. Anim. Indus. 36 pp., illus. Revised, February 1933. [Mimeographed.]

The sample was tightly wrapped in a 5-cm square of filter cloth, placed on a perforated aluminum shelf, 1 cm high, in the pressometer tray, and kept under a pressure of 250 pounds for 5 minutes. Previous work had shown that during this period most of the nitrogen is removed (4). It was necessary to strain the press fluid when using raw meat in order to remove particles of fibers which were forced through the cloth by pressure. A saturated filter cloth square was used for this purpose.

Ten cubic centimeters of press fluid were collected for the determination of moisture, total nitrogen, and noncoagulable nitrogen in the press fluid. The fluid was kept at a temperature of 4° C. until analyzed. The percentage of moisture and of total and noncoagulable nitrogen in press fluid was determined by the method of the Association of Official Agricultural Chemists (1). The percentage of coagulable nitrogen was obtained by the difference between the means of duplicate analyses of total and noncoagulable nitrogen.

EXPERIMENTAL DATA

THE HOMOGENEITY OF SEMITENDINOSUS BEEF MUSCLE FOR PRESS-FLUID DETERMINATION

In a series of preliminary experiments portions of the semitendinosus beef muscle 5 cm apart were tested for uniformity of quantity and composition of press fluid, five muscles being used.

Three thermometers, 5 cm apart and approximately 5 cm from either end, were inserted in each muscle, which was cooked until the center thermometer registered 58° C., the end thermometers ranging between 59° and 60°. After the muscle was removed from the oven the rise in temperature varied from 1° to 3°. Press-fluid determinations were made on duplicate samples of slices taken from the anterior, center, and posterior parts of the muscle. Cooking losses were obtained from the roasts.

The results of this study showed that the differences in percentage of press fluid in each portion were so slight as to be negligible. The percentage of cooking losses likewise showed a very small deviation among the roasts.

A chemical analysis to determine the moisture, total nitrogen, and ether-extract content of the press fluid from the different portions was made. The composition of the press fluid in the muscle was found to be within the limits of experimental error. The ether extract was so small a percentage that this analysis was omitted in later work.

According to these analyses the semitendinosus beef muscle was uniform in chemical composition as well as in press-fluid yield; therefore, two comparable roasts were used from the same muscle.

PRESS FLUID IN SEMITENDINOSUS BEEF MUSCLE HEATED TO 58° AND 75° C.

Duplicate samples averaging 1.5 g were taken for the determination of press fluid from roasts from the same muscle. The anterior section was heated to 58° C. and the posterior to 75°. The press fluid in muscles heated to 58° averaged 54.175 percent, with a standard deviation of 2.14; and that of muscles heated to 75° averaged 43.094 percent, with a standard deviation of 3.25.

¹ The temperatures 58° and 75° C. were reached in the oven. After removal, the rise in temperature of the 58° roast varied from 1° to 3°, but the temperature of the 75° roast remained constant.

The grams of press fluid per gram of dry matter were obtained by bringing the pressed sample to constant weight. The data obtained showed that the press fluid per gram of dry matter averaged 2.3186, with a standard deviation of 0.201 for the 58° muscles; and 1.4605, with a standard deviation of 0.248, for the 75° muscles.

Applying Fisher's (?) analysis of variance to the data on the percentages of press fluid and the grams of fluid per gram of dry matter, it was found that variation due to temperature was much larger than that due to error.

Data on both percentages of press fluid and grams of fluid per gram of dry matter show that there is a larger quantity of press fluid in muscle heated to 58° C. than in that heated to 75°.

When Fisher's (?) analysis of variance was applied to the data, a tendency was noted for the raw beef muscle to differ from the muscle heated to 58° and 75° C. in moisture, total nitrogen, and noncoagulable nitrogen (table 2).

TABLE 2.—Means in percentage of moisture, total nitrogen, noncoagulable nitrogen and coagulable nitrogen in press fluid from raw beef muscle and the same heated to 58° and 75° C.

Sample no.	Moisture			Total nitrogen			Noncoagulable nitrogen			Coagulable nitrogen		
	Raw	58°	75°	Raw	58°	75°	Raw	58°	75°	Raw	58°	75°
1.....	87.85	91.04	92.83	1.68	1.02	0.76	0.55	0.70	0.87	1.14	0.32	0.09
2.....	87.71	91.94	93.55	1.82	1.04	.74	.42	.55	.58	1.41	.48	.16
3.....	90.10	91.19	95.25	1.32	1.11	.64	.46	.70	.49	.86	.42	.04
4.....	88.61	91.61	93.57	1.57	1.05	.70	.50	.59	.66	1.07	.46	.01
5.....	88.25	90.63	94.05	1.63	1.08	.57	.47	.66	.51	1.16	.48	.06
6.....	89.98	90.14	93.65	1.36	1.18	.60	.48	.76	.62	.87	.42	.04
7.....	88.80	92.69	93.46	1.54	.89	.64	.43	.59	.57	1.11	.31	.08
8.....	87.68	90.45	93.68	1.25	1.05	.67	.48	.68	.63	.77	.47	.04
9.....	90.56	91.73	93.79	1.21	1.01	.59	.46	.63	.54	.75	.37	.05
10.....	87.43	91.16	92.16	1.61	1.06	.66	.49	.56	.61	1.12	.49	.05
11.....	87.45	91.53	93.66	1.58	1.03	.59	.48	.53	.54	1.10	.49	.05
12.....	87.38	92.23	94.26	1.68	.92	.60	.47	.63	.56	1.23	.80	.03
13.....	89.65	91.40	93.73	1.48	1.03	.68	.47	.66	.63	1.03	.49	.05
14.....	88.52	91.09	93.67	1.26	.89	.55	.39	.64	.50	.85	.24	.05
15.....	86.94	91.02	92.53	1.67	.86	.65	.41	.66	.65	1.24	.20	.01
16.....	90.16	91.80	93.33	1.26	.93	.64	.40	.61	.60	.87	.31	.05
Mean.....	88.56	91.37	93.57	1.49	1.01	.64	.46	.63	.58	1.04	.39	.05

The means of the different analyses show three distinct trends in the composition of the press fluid at each temperature. The moisture content of the press fluid varies directly with the interior temperature, being 93.57 percent in muscle heated to 75° C., and 91.37 percent in muscle heated to 58°. Raw muscle has the lowest percentage, 88.56. As the muscle proteins coagulate with increased temperature, the coagulable nitrogen fraction is removed, thus yielding a less concentrated press fluid.

The percentage of total nitrogen shows an inverse relationship with the temperature, 0.64 percent in the 75° muscle, and 1.01 percent in the 58° muscle. The raw muscle has the highest percentage, 1.49. The removal of the heat-coagulable nitrogen with increased temperature brings about this relationship.

The averages for the percentage of coagulable nitrogen in the press fluid follow the same order as for the total nitrogen, 1.04 percent for the raw, 0.39 percent for the 58°, and 0.05 percent for the 75°.

Coagulable nitrogen varies consistently with increase in temperature, little being present at 75°. The small quantity of coagulable nitrogen at 75° indicates that coagulation is practically complete at that temperature. The noncoagulable nitrogen content averages 0.46 percent for the press fluid from the raw muscle, 0.63 for the 58°, and 0.58 percent for the 75°.

These values indicate that the noncoagulable nitrogen from the 58° and that from the 75° muscle are only slightly different. Enzymes may be the influencing factor. The increased enzyme activity at 58° C. releases additional amino nitrogen which analyzes as non coagulable nitrogen. The higher temperature of 75° inhibits enzyme activity, thus decreasing the yield of noncoagulable nitrogen.

PRESS FLUID AND COOKING LOSSES

Press fluid and total cooking losses were obtained for roasts cooked to 58° and 75° C. interior temperatures. These data are given in table 3 (32 pairs of observations).

TABLE 3.—Press fluid and total cooking losses in semitendinosus beef muscle heated to 58° and to 75° C.

58° C.			75° C.		
Roast no.	Press fluid	Cooking losses	Roast no.	Press fluid	Cooking losses
	Percent	Percent		Percent	Percent
1.....	58.346	9.759	2.....	47.872	25.719
3.....	56.416	10.836	4.....	44.315	24.752
5.....	53.347	19.230	6.....	41.950	30.688
7.....	52.693	9.136	8.....	42.675	24.483
9.....	54.203	15.000	10.....	51.218	32.626
11.....	54.946	13.810	12.....	41.830	32.409
13.....	56.282	12.376	14.....	41.088	34.708
15.....	52.960	11.281	16.....	44.415	31.463
17.....	53.274	11.807	18.....	41.968	30.857
19.....	55.059	12.619	20.....	46.214	30.081
21.....	54.910	10.976	22.....	40.612	30.417
23.....	53.875	10.304	24.....	41.858	35.345
25.....	55.534	11.491	26.....	42.262	31.330
27.....	55.363	13.400	28.....	39.726	32.200
29.....	50.814	14.050	30.....	38.950	37.846
31.....	54.284	11.258	32.....	45.356	30.045
33.....	53.185	19.001	34.....	39.184	36.657
35.....	55.814	14.167	36.....	37.890	34.342
37.....	55.308	12.224	38.....	48.843	29.768
39.....	51.284	13.907	40.....	42.382	29.791
41.....	54.060	13.062	42.....	45.410	28.831
43.....	53.380	11.811	44.....	39.066	37.021
45.....	55.176	15.385	46.....	43.843	32.821
47.....	55.806	10.938	48.....	44.404	32.688
49.....	51.410	11.111	50.....	45.345	26.038
51.....	55.416	14.471	52.....	46.116	34.156
53.....	49.222	13.937	54.....	42.768	29.392
55.....	53.074	9.388	56.....	45.703	22.303
57.....	56.65	11.957	58.....	45.602	31.070
59.....	54.094	14.566	60.....	46.692	27.588
61.....	54.887	10.358	62.....	47.006	25.086
63.....	52.782	11.849	64.....	41.786	33.108

The value for the correlation coefficient r is -0.5331 when correlating percentage of press fluid and total cooking losses from the 75° C. roasts. This value is highly significant, since the least highly significant value ($P=0.01$) for r is 0.4487 .

The value for r in the correlation between percentage cooking losses and press fluid in the roasts cooked to 58° C. is -0.1823 , and

the least significant value ($P=0.05$) for r is 0.3494. No significant relationship is indicated between total cooking losses and press fluid in roasts cooked to 58° . According to McCance and Shipp (12), muscle tissue shortens without a change in volume or loss of weight when heated to 40° . However, at temperatures above 60° there is a loss of weight caused by increased shrinkage of the meat proteins, causing expression of the meat juices. This may explain the inverse relationship observed when muscle is heated to 75° and the fact that there is no definite relationship at 58° .

SUMMARY

From this study the following observations on the effect of interior temperature upon the semitendinosus muscle of beef can be made on the basis of statistical analysis.

The ratio of press fluid to dry matter is greater in muscle heated to 58° C. than in that heated to 75° , the value for grams of press fluid per gram of dry matter being 2.319 for the 58° muscle and 1.46 for the 75° .

Approximately 11 percent more press fluid is found in the muscle heated to 58° than in that heated to 75° .

Chemical analysis of the press fluid showed:

The moisture content of press fluid varies directly with the interior temperature, the raw having less moisture than the heated muscle.

An inverse relationship exists between the total nitrogen content of press fluid and the interior temperature, the raw having more total nitrogen.

There is comparatively little difference in the noncoagulable nitrogen in press fluid from muscles heated to 58° and 75° C., the raw having less than the heated muscle.

The coagulable nitrogen fraction in press fluid varies inversely with the interior temperature.

An inverse relationship exists between the percentage of press fluid and the total cooking losses in muscle heated to 75° C. No relationship is indicated between the percentage of press fluid and the total cooking losses in muscle heated to 58° .

LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Compiled by the committee on editing methods of analysis . . . Ed. 3, 593 pp., illus. Washington, D. C.
- (2) BIGELOW, W. D., and COOK, F. C.
1908. MEAT EXTRACTS AND SIMILAR PREPARATIONS, INCLUDING STUDIES OF THE METHODS OF ANALYSIS EMPLOYED. U. S. Dept. Agr., Bur. Chem. Bull. 114, 56 pp.
- (3) BOTAZZI, E.
1912. RECHERCHES SUR LA CONSTITUTION PHYSIQUE ET LES PROPRIETES CHIMICO-PHYSIQUES DU SUC DES MUSCLES LISSES ET DES MUSCLES STRIES. Arch. Internat. Physiol. 12: 243-246, 409-448.
- (4) CHILD, A. M., and BALDELLI, M.
1934. PRESS FLUID FROM HEATED BEEF MUSCLE. Jour. Agr. Research 48: 1127-1134, illus.
- (5) CLINE, J. A., and GODFREY, R. S.
1927. A STUDY OF TEMPERATURE AND TIME OF COOKING ON THE QUALITY AND PALATABILITY OF MEAT. Mo. Agr. Expt. Sta. Bull. 256: 74-75.

-
- (6) CLINE, J. A. TROWBRIDGE, E. A., FOSTER, M. T., and FRY, H. E.
1930. HOW CERTAIN METHODS OF COOKING AFFECT THE QUALITY AND PALATABILITY OF BEEF. *Mo. Agr. Expt. Sta. Bull.* 293, 40 pp., illus.
- (7) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.
- (8) GRINDLEY, H. S., and EMMETT, A. D.
1905. STUDIES ON THE INFLUENCE OF COOKING UPON THE NUTRITIVE VALUE OF MEATS AT THE UNIVERSITY OF ILLINOIS, 1903-1904. U. S. Dept. Agr., Off. Expt. Stas. Bull. 162, 230 pp.
- (9) ——— and MOJONNIER, T.
1904. EXPERIMENTS ON LOSSES IN COOKING MEATS, 1900-1903. U. S. Dept. Agr., Off. Expt. Stas. Bull. 141, 95 pp.
- (10) HALLIDAY, E. G., NOBLE, I. T., and KLAAS, H. K.
1934. RESEARCH STUDIES ON TENDERNESS AND JUICINESS OF COOKED MEAT. *Jour. Home Econ.* 26: 238-242.
- (11) LEACH, A. E.
1909. FOOD INSPECTION AND ANALYSIS. FOR THE USE OF PUBLIC ANALYSTS, HEALTH OFFICERS, SANITARY CHEMISTS, AND FOOD ECONOMISTS. Ed. 2, rev. and enl., 954 pp., illus. New York.
- (12) McCANCE, R. A., and SHIPP, H. L.
1933. THE CHEMISTRY OF FLESH FOODS AND THEIR LOSSES ON COOKING. [Gt. Brit.] Med. Research Council Spec. Rept. Ser. 187, 146 pp., illus.
- (13) SPRAGUE, E. C., and GRINDLEY, H. S.
1907. A PRECISE METHOD OF ROASTING BEEF. *Ill. Univ. Studies* v. 2, no. 4, 37 pp., illus.
- (14) WILEY, H. W.
1911. FOODS AND THEIR ADULTERATION; ORIGIN, MANUFACTURE, AND COMPOSITION OF FOOD PRODUCTS; INFANTS' AND INVALIDS' FOODS; DETECTION OF COMMON ADULTERATIONS AND FOOD STANDARDS. Ed. 2, rev. and enl., 641 pp., illus. Philadelphia.

THE DIFFUSIBLE CALCIUM IN THE SERUM OF LAYING AND NONLAYING HENS¹

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INTRODUCTION

The calcium metabolism of fowls is of considerable theoretical interest because of their ability to absorb and to deposit in the eggshell a large amount of calcium. It is, also, of interest that the hen is able to tolerate readily, as compared with mammals, an extremely high level of calcium in the blood. In an effort to obtain facts which might serve to explain this high tolerance for calcium, determinations were made of the diffusible and nondiffusible calcium in the blood serum of laying and of nonlaying hens. It seemed particularly desirable in these studies to ascertain whether there were any changes in the relative amounts of these two calcium fractions when the hen passed from a nonlaying into a laying status and vice versa. Therefore, the determinations were made on serum from a small group of hens before they began to lay and after they began to lay.

REVIEW OF LITERATURE

Since the completion of this work, reports on the same subject have appeared from other laboratories. Correll and Hughes (3),² comparing cocks, laying hens, and nonlaying hens, found the ultrafiltrable calcium of the serum to be fairly constant at a value of 6.4 mg, although the total calcium varied from 11.7 to 25.1 mg per 100 cc. Their analyses were carried out according to the method of Nicholas (?), with cellophane as the semipermeable membrane. Laskowski (5), using collodion membranes, found the ultrafiltrable calcium to be 8.0 mg per 100 cc in the case of both laying and nonlaying hens. However, this value was obtained on plasma rather than on serum. Benjamin and Hess (1), using a collodion membrane and relatively low pressure differences, obtained a value of 6.8 mg per 100 cc of serum for laying hens. It is unfortunate, for the purposes of comparison, that the methods used in these investigations and in ultrafiltration and diffusion studies in general have varied considerably. However, the results obtained by different investigators on avian serum are in essential agreement.

EXPERIMENTAL METHOD

The methods available for this type of study may be classified in three groups: (1) Ultrafiltration, in which the serum is forced through a semipermeable membrane by high or low pressure; (2) pressure

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² Reference is made by number (italic) to Literature Cited, p. 667.

dialysis, in which the sample is separated from pure water or a salt solution by a semipermeable membrane, a difference in pressure being maintained between the two sides of the membrane; and (3) ordinary dialysis. The second method was chosen for these studies since it appeared to lend itself readily to the handling of small samples.

The apparatus used for the pressure dialysis of the serum was essentially that described by Moritz (6). The negative pressure was obtained by means of a suction pump and was regulated at $150\text{ mm} \pm 10\text{ mm}$ of mercury by means of a mercury escape valve. It was found necessary to include a large reservoir in the circuit to avoid wide fluctuations in pressure.

Collodion sacs were prepared according to a method based on the work of Farmer (4) and Brown (2). A solution of 10-percent Parlodion in equal parts of absolute ethyl alcohol and diethyl ether was used. A test tube of appropriate size was filled with collodion, emptied, and drained for exactly 1 minute, as described by Farmer (4). Then, with the open end of the test tube up, the film was dried for exactly 1 minute by lowering a capillary tube, to which suction was applied, nearly to the bottom of the test tube. The test tube was then immersed in 95-percent ethyl alcohol and allowed to remain for at least 5 minutes. The alcohol was applied to harden the membrane and to give high permeability. Several investigators have used alcohol for this purpose, particularly in the preparation of membranes of graded permeability; the higher the concentration of alcohol used for hardening, the greater the permeability. The sacs were then removed from the tubes, washed with distilled water, and preserved for future use in normal saline solution. Chloroform was added as a preservative. It is important that the sacs should not be allowed to dry since drying greatly reduces the permeability.

In carrying out a determination, the following procedure was adopted: Three cubic centimeters of distilled water was measured into a 15-cc centrifuge tube and the tube and contents weighed. A sac from the saline solution was washed with distilled water and dried inside and out with filter paper, and 2 cc of serum was then measured into it. In practice it was found easiest to slip the sac onto its supporting stopper before adding the serum, and then to add the serum by inserting the tip of the pipette through the hole of the stopper. The sac was then secured to the stopper by means of an encircling thread and the whole pushed onto the glass tube which opened to the atmosphere. These operations had to be carried out quickly to prevent any lessening of the permeability of the sac by drying. After the complete apparatus had been assembled and when suction was first applied, the sac was held above the level of the water in order to observe possible leaks. It was then lowered so that the level inside the sac was just below that on the outside. The dialysis was carried on for 4 hours, the sac being raised occasionally to maintain the relative levels of the liquids.

At the end of the dialysis the sac was again raised above the level of the outside liquid and the suction slowly released. The apparatus was separated, care being taken to transfer as much as possible of the liquid adhering to the outside of the sac to the centrifuge tube. The latter was then weighed, the gain in weight being the measure of the water which had left the sac. No correction was found to be necessary for the amount of water adhering to the sac since several trials showed

it to be negligible. The outside of the sac was then washed into the tube containing the dialysate, and the solution within the sac was washed out into a second centrifuge tube. Calcium was determined in both of these by the method of Kramer and Tisdall as modified by Tisdall (8). From these results the diffusible calcium was calculated in the way described by Updegraff, Greenberg, and Clark (9). The total calcium concentration was obtained by adding the amount of calcium in the dialysate to that which remained in the sac. The accuracy of this calculation was found to be within the limits of error of the method for calcium determination.

Six nonlaying hens which later began to lay were employed in this experiment. Blood samples of about 30 cc were obtained from the heart by means of a hypodermic needle and syringe at approximately monthly intervals. The blood serum was separated by centrifuging.

RESULTS AND DISCUSSION

Since trouble was experienced in preparing highly permeable membranes, a number of determinations were carried out on a solution of $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ containing 18 mg of calcium per 100 cc. Under these conditions, 90.9 ± 1.4 percent of the calcium was diffusible through the various membranes. While theoretically 100 percent of the calcium in an entirely inorganic solution should be diffusible, it is doubtful whether this would be the case under the conditions of this experiment. Thus, it is probable that a greater degree of diffusibility would have been found had the dialysis period been longer. Furthermore, duplicate tests on serum showed close agreement even though the permeability of the particular membranes used in the tests varied widely when used with the inorganic calcium solution.

The results obtained on the dialysis of serum are summarized in table 1. From these figures it may be seen that although the total calcium level of the serum varied from 10.6 to 39.0 mg per 100 cc, the diffusible calcium was maintained within a much narrower range, all the values except two falling between 4.2 and 6.7, with an average for all determinations of 5.3 ± 0.17 mg. This value is lower than the 6.4 reported by Correll and Hughes (3) or the 6.8 reported by Benjamin and Hess (1). The differences may be due to the fact that the aforementioned investigators used ultrafiltration methods, while the method employed in these experiments was pressure dialysis. The significantly higher value, 8.0 mg, which Laskowski (5) obtained on plasma is not strictly comparable with the results obtained on serum. It may be concluded, therefore, that for any given method of analysis the so-called "ultrafiltrable" or diffusible calcium of fowl serum shows a high degree of constancy which is quite independent of the total calcium concentration of that serum. Benjamin and Hess (1) and others have shown that this is not true in cases of other species in which the level of calcium in the blood has been elevated artificially or pathologically. It is apparent, also, that slight changes in the procedure, at least in this particular type of analysis, may give constant differences in results.

TABLE 1.—Total and diffusible calcium of hen's serum

Bird no	Month and day of analysis	Condition of bird	Determination ¹	Total calcium †		Diffusible calcium †	
				Range	Mean	Range	Mean
			Number	Mg	Mg	Mg	Mg
123	Jan. 12	Nonlaying.....	4	9.9-11.3	10.6	6.3-6.6	6.5
	Jan. 26	Laying.....	5	20.2-21.7	21.3	6.1-6.5	6.3
	Feb. 16	do.....	2	25.5-25.5	25.5	5.0-5.4	5.2
	May 3	do.....	3	17.3-20.0	18.9	3.9-4.8	4.2
274	Jan. 12	Nonlaying.....	5	11.3-11.9	11.6	5.8-6.5	6.2
	Jan. 27	Laying.....	5	23.5-24.7	23.8	5.5-6.6	6.0
	Feb. 16	Nonlaying.....	4	11.8-12.1	11.9	3.9-4.9	4.5
	Jan. 15	do.....	4	11.9-12.3	12.1	6.5-6.8	6.7
177	Jan. 29	Laying.....	4	38.2-39.9	39.0	5.1-6.2	5.6
	Feb. 26	do.....	5	16.5-20.4	18.8	3.1-4.6	3.7
	Jan. 19	do.....	5	24.8-26.5	25.9	5.7-7.4	6.5
	Feb. 9	do.....	4	18.5-19.3	18.9	4.3-5.1	4.6
372	Apr. 19	do.....	4	21.8-22.1	21.9	4.2-4.4	4.3
	Jan. 19	Nonlaying.....	4	12.5-13.4	12.7	5.6-6.8	6.1
	Feb. 9	Laying.....	4	17.2-18.9	17.9	6.1-6.7	6.4
	Apr. 19	do.....	3	16.5-16.5	16.5	4.9-5.2	5.0
923	Jan. 21	do.....	5	15.3-16.1	15.6	5.1-6.1	5.7
	Feb. 11	Nonlaying.....	2	11.7-12.7	12.2	2.5-2.9	2.7
	May 3	Laying.....	3	13.4-14.5	14.1	4.3-5.5	5.1
	Jan. 5	Nonlaying; on rachitogenic diet.....	6	12.6-13.3	12.9	7.9-8.5	8.2

¹ Per 100 cc of serum

As indicated earlier in this paper, the birds were in a nonlaying condition at the beginning of the experiment, but each one began to lay before or soon after the first set of analyses had been made. In some instances there occurred a further change back to the nonlaying condition prior to the last analysis. Although in each case the total calcium increased markedly with the advent of the laying period, in no case was there a significant change in the value of the diffusible calcium. Furthermore, the average value for all analyses made while the birds were not laying, 5.4 ± 0.42 mg per 100 cc is not significantly different from the value 5.3 ± 0.17 mg per 100 cc obtained when they were laying. The identity of these values seems particularly significant because all the values were determined on serum from the same group of birds. It is generally agreed that the nondiffusible calcium of serum exists in some kind of combination with the protein. Benjamin and Hess (1) have further divided the diffusible and nondiffusible fractions according to whether or not they are absorbed by dry BaSO_4 . They find, as do others, that a rise in the total calcium of the blood is generally accompanied by a rise in serum protein. The possibility that the nondiffusible calcium exists as a calcium-protein complex may explain the tolerance of laying hens for an extremely high level of blood calcium. It is not known whether the calcium in this complex is available for the formation of eggshell, but apparently it has little toxic activity.

Bird no. 10 had been on a rachitic diet for 9 months previous to the assay. It was killed by decapitation and the blood sample collected during the subsequent bleeding. The value 8.2 mg per 100 cc of serum for the diffusible calcium, while it may be due to the method of bleeding, is believed to be indicative of the disturbed calcium metabolism caused by the rachitic diet.

SUMMARY

By the use of collodion membranes, the diffusible calcium of the serum of nonlaying hens was found to be 5.4 ± 0.42 and that of the same hens in the laying condition 5.3 ± 0.17 mg per 100 cc of serum. No difference was observed in the level of diffusible calcium when the birds changed from a nonlaying to a laying condition or vice versa. The nondiffusible calcium in the serum rose from a value of 6.4 ± 0.53 mg per 100 cc for nonlaying birds to a value of 16.1 ± 1.17 mg per 100 cc for the same birds in the laying condition.

LITERATURE CITED

- (1) BENJAMIN, H. R., and HESS, A. F.
1933. THE FORMS OF THE CALCIUM AND INORGANIC PHOSPHORUS IN HUMAN AND ANIMAL SERA. III. A COMPARISON OF PHYSIOLOGICAL AND EXPERIMENTAL HYPERCALCEMIA. *Jour. Biol. Chem.* 103: 629-641.
- (2) BROWN, W.
1915. ON THE PREPARATION OF COLLODION MEMBRANES OF DIFFERENTIAL PERMEABILITY. *Biochem. Jour.* 9: [591]-617, illus.
- (3) CORRELL, J. T., and HUGHES, J. S.
1933. THE RELATION OF FILTRABLE TO NONFILTRABLE CALCIUM IN CHICKEN BLOOD. *Jour. Biol. Chem.* 103: 511-514.
- (4) FARMER, C. J.
1917. A METHOD FOR THE PREPARATION OF UNIFORM COLLODION MEMBRANES FOR DIALYSIS. *Jour. Biol. Chem.* 32: 447-453, illus.
- (5) LASKOWSKI, M.
1933. ÜBER DEN CALCIUMZUSTAND IM BLUTPLASMA DER HENNE. *Biochem. Ztschr.* 260: 230-240.
- (6) MORITZ, A. R.
1925. THE EFFECT OF ULTRAVIOLET IRRADIATION ON THE STATE OF THE SERUM CALCIUM. *Jour. Biol. Chem.* 64: 81-89, illus.
- (7) NICHOLAS, H. O.
1932. DIFFUSIBLE SERUM CALCIUM BY HIGH PRESSURE ULTRAFILTRATION. *Jour. Biol. Chem.* 97: 457-464, illus.
- (8) TISDALL, F. F.
1923. A NOTE ON THE KRAMER-TISDALL METHOD FOR THE DETERMINATION OF CALCIUM IN SMALL AMOUNTS OF SERUM. *Jour. Biol. Chem.* 56: 439-441.
- (9) UPDEGRAFF, H., GREENBERG, D. M., and CLARK, G. W.
1926. A STUDY OF THE DISTRIBUTION OF THE DIFFUSIBLE AND NONDIFFUSIBLE CALCIUM IN THE BLOOD SERA OF NORMAL ANIMALS. *Jour. Biol. Chem.* 71: 87-117, illus.

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FACTORS INVOLVED IN THE APPLICATION OF FORM-CLASS VOLUME TABLES¹

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INTRODUCTION

A method of constructing generalized taper charts and volume tables, based upon a formula for the stem curve and developed in its general principles from the form-class system used in Sweden, has been outlined in a previous paper by the writer (2).² In applying these generalized taper charts and volume tables to the measurement of stands of timber of any given species, the necessary steps are: (1) To study the variability of the form quotients of the trees in a stand in order to ascertain whether a single average form quotient may be used for the entire stand; (2) to work out a method of determining the average form quotient of the stands to be estimated in the field so that the proper form-class table may be selected for each set of conditions; and (3) to study the relation between normal diameter inside bark at breast height (4.5 feet above the ground) and the actual breast-height diameter for the species in question.

Some suggestions in regard to each of these three processes as well as references to the earlier literature were included in the previous paper. Since the problems referred to are inherent in the timber itself and are not peculiar to the form-class system alone, they enter into the application of volume tables of any sort and so merit intensive analysis. In this paper are presented the results of detailed statistical studies of these questions for red spruce (*Picea rubra* Link), white spruce (*Picea glauca* (Moench) Voss), and balsam fir (*Abies balsamea* (L.) Mill.) in the Northeast. Wright (9) studied these questions for the coniferous species of eastern Canada, but his results are not directly comparable to those given in this paper, primarily because he did not eliminate the effect of butt swell on diameter at breast height before determining form quotient. Furthermore, his data for spruce and fir were obtained from not more than eight stands for any one species, so that his method of approach was necessarily quite different. Gevorkiantz and Hosley (4), in their study of the form and development of white pine stands in Massachusetts, evolved a crown index, or measure of diameter of crown in relation to breast-high diameter of the tree, which when used in conjunction with the relative length of the crown provided a satisfactory basis for estimating form quotient.

Perhaps the most thorough investigations of variations in bark thickness in relation to form-quotient and volume estimation were those made by Heijbel (6) for Scotch pine in Sweden. He found

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² Reference is made by number (italic) to Literature Cited, p. 713.

that the relative thickness of bark at breast height varied with the diameter of the tree, while bark thickness measured at one-tenth of total height was constant in all except the smallest size classes. He also found that for a given diameter class the shorter trees had relatively thicker bark. Scotch pines of low form class also appeared to have relatively thicker bark than those of higher form class. Heijbel proposes that bark thickness and volume determination be based on measurements at one-tenth the total height of the tree instead of at the fixed breast-height point.

In the study of the application of form-class methods to oak in Sweden, Hedeby (5) observed that form quotient of standing timber might be estimated from either the form point or relative length of crown, but that better estimates were obtained when diameter breast high was taken into consideration along with either of these factors.

In none of the studies reported has multiple-correlation technic been employed in attempting to evaluate the effect of various factors on form quotient, bark thickness, or butt swell.

BASIS AND METHODS OF STUDY

FIELD WORK

DISTRIBUTION OF DATA

In order to study the effect of such stand factors as age, site index, density, and average height upon variations in average form quotient, or difference between normal diameter inside bark and actual diameters at breast height, it is necessary to obtain taper measurements on enough trees to give a satisfactory average in each of a number of stands representing the entire range of conditions to be considered. For this reason it was desirable to get samples from as many stands as possible, keeping the number of trees in each stand as low as was consistent with satisfactory accuracy of the averages. The desired degree of accuracy was set at a standard error of ± 1 unit in the average form quotient of the stand.

Data previously presented (2) and substantiated in this study indicate that the standard deviation of form quotients within a stand averages about 4.5 units. On this basis measurements of 20 trees are required to obtain an average with the desired accuracy of ± 1 unit. Increasing the number of trees to 30 reduces the standard error of the average from 1.0 to 0.82 units, and 40 trees only brings it down to 0.71 units, so that the gain in accuracy from the use of a larger number of trees must be balanced against the extra time required for their measurement.

Primary emphasis was placed on obtaining measurements in stands of even-aged second-growth timber, which lends itself much better than old growth to the evaluation of such factors as age, density, etc. Enough data were secured from old-growth stands, however, to afford a basis for studying the extent of variations of form within the principal types of the region and to indicate average form quotients that may prove generally applicable to these types, as well as to establish the relation between normal and actual diameters at breast height for timber of this character.

Measurements of 2,070 trees were taken in 74 stands scattered over northern New England and New York, covering a wide range of

mixture, age, site, and density. In stands in which any of the three species under study were present in mixture, samples were taken of each species. Except that obviously deformed or abnormal individuals were eliminated and an effort was made to cover the range of sizes present, trees were taken at random in each stand. In addition, data from 119 trees, measured by Meyer (8) as samples in connection with his study of yield of second-growth spruce and fir in the Northeast, were incorporated into this study. The distribution of these measurements of 2,189 trees among the various species and types of growth is shown in table 1.

TABLE 1.—Basis for statistical analyses of trees

Type of growth	Red spruce	White spruce	Balsam fir	All species
Even aged.....	Number 664	Number 601	Number 347	Number 1,612
Old growth.....	345	-----	232	577
Total.....	1,009	601	579	2,189

The ranges of age, site, and density covered by the samples for each species from the 74 different stands are shown in tables 2, 3, and 4.

TABLE 2.—Distribution of sample stands by age classes

Age (years)	Red spruce	White spruce	Balsam fir	Age (years)	Red spruce	White spruce	Balsam fir
	Number	Number	Number		Number	Number	Number
10-19.....	1	1	-----	100-109.....	1	-----	1
20-29.....	1	3	2	110-119.....	-----	-----	-----
30-39.....	1	9	4	120-129.....	-----	-----	-----
40-49.....	8	8	6	130-139.....	1	-----	1
50-59.....	3	3	1				
60-69.....	6	3	1	Even-aged stands.....	31	28	17
70-79.....	5	1	1	Old-growth stands.....	17	0	11
80-89.....	2	-----	-----	Total.....	48	28	28
90-99.....	2	-----	-----				

TABLE 3.—Distribution of sample stands by site classes

Site index ¹	Even-aged			Old-growth	
	Red spruce	White spruce	Balsam fir	Red spruce	Balsam fir
	Number	Number	Number	Number	Number
30-34.....	1	-----	-----	1	-----
35-39.....	3	-----	1	2	2
40-44.....	5	2	5	2	2
45-49.....	9	7	2	2	2
50-54.....	8	9	2	6	5
55-59.....	1	6	4	2	1
60-64.....	3	4	3	2	-----
65-69.....	1	-----	-----	-----	-----
Total.....	31	28	17	17	13

¹ Based on average height of dominant trees and red spruce site-classification curves of Meyer (8). ² Site index of stands without red spruce expressed on basis of red spruce height growth by reducing white spruce heights 7 percent and balsam fir heights 9 percent.

TABLE 4.—Distribution of sample stands by total number of trees per acre

Trees per acre (number)	Even-aged			Old-growth	
	Red spruce	White spruce	Balsam fir	Red spruce	Balsam fir
	Number	Number	Number	Number	Number
100-199.....				1	
200-299.....		1		6	2
300-399.....	2	2	2	3	3
400-499.....	4	3	2		
500-599.....	3	2		1	1
600-699.....	4	4	2	2	2
700-799.....	5	1	1	1	1
800-899.....		2	1	1	1
900-999.....	2	4	2		
1,000-1,499.....	6	5	1	1	1
1,500-1,999.....	3	2	3	1	
2,000-2,499.....	2		1		
2,500-2,999.....		1	1		
3,000-3,999.....			1		
4,000-4,999.....		1			
Total.....	31	28	17	17	11

MEASUREMENTS TAKEN

In each stand in which measurements were taken a tally was obtained, either on a random strip survey or a rectangular sample plot, of the number of trees of each species by diameter classes. Descriptive notes covering history and general condition of the stand, underbrush, ground cover, soil, slope, injuries, site, etc., were also taken.

On each tree to be measured an estimate of form-point height as a percentage of total height was obtained with the aid of a hypsometer of the Christen type. Notes of crown class and shape of tip—flat, rounded, or pointed—were also made. In stands where the trees were being cut, total height and crown length were obtained with the tape as soon as the trees were felled; in stands where no cutting was being done, total height was measured with a Faustman hypsometer, and crown length as percentage of total height was measured with a Christen hypsometer. Heights were read to nearest foot. On each tree, age was obtained either from ring count on stump or from increment borings, usually taken 2.5 feet from the ground. Diameter measurements were obtained at stump (if cut), at 2.5 feet from ground, at breast height, and at one-twentieth, one-tenth, two-tenths, three-tenths, and each succeeding tenth of the stem between breast height and top as far as it was practicable to measure. On trees that were not cut, the taper measurements were not taken further than the seventh tenth above breast height. Measurements were taken with diameter tape to nearest tenth of an inch. At each point of diameter measurement thickness of bark was ascertained either with a hatchet and rule or by the use of a Swedish bark borer.

In a number of stands in which measurements were taken while pulpwood peeling operations were in progress diameter measurements inside bark were obtained directly from the peeled stems. In all such cases, breast-height diameter outside bark was taken and bark thickness was measured with the borer before peeling to provide a check on the accuracy of the bark borer.

ACCURACY OF BARK-THICKNESS MEASUREMENTS

Analysis of 156 measurements taken on red spruce at time of peeling indicated that with the Swedish bark borer the thickness of bark of this species is underestimated, especially in old-growth timber. The standard error of the estimate of diameter inside bark from tape measurement outside bark reduced by use of the Swedish bark borer was ± 0.16 inch, and the average error was $+0.07$ inch. In 83 cases the errors in diameter were positive; in 25, negative; and in 48, no errors were indicated. The diameters included ranged from 6 to 23 inches.

From 33 balsam fir trees measured in this way no consistent bias was observed as a result of the use of the Swedish bark borer. The standard error of the inside bark diameter estimates was ± 0.08 inch.

Because a change from hatchet and rule to Swedish bark borer for determination of bark thickness was made after the first season's field work in this study, and because considerable differences appeared in the bark-thickness and butt-swell relations obtained from the data for the first 2 years, it seemed necessary to investigate the relative accuracy of the two methods of measuring bark thickness.

Measurements were obtained on 162 red spruce trees selected from a variety of stands in western Massachusetts, including both old growth and second growth. Diameters outside bark were taken with diameter tape. The bark of each tree was first measured with the Swedish bark borer at two points as nearly opposite as possible. The bark at points of measurement was then notched with a hatchet, and an average reading to nearest 0.05 inch was made with a steel rule.

The difference in double bark thickness was calculated for each point of measurement. These differences were grouped and averaged according to the diameters of the trees.

For the entire sample of 324 measurements the bark borer gave a double bark thickness averaging 0.077 inch greater than that given by the hatchet and rule. The difference tends to increase with the diameter of the trees, the coefficient of correlation being 0.456 ± 0.044 . The average difference does not exceed 0.1 inch of double bark until trees are 13 inches d. b. h.,³ and does not account for more than one-third of the spread noted in the bark-thickness and butt-swell relations of the data for the first 2 years of this study. On the whole, therefore, no importance need be attached to the difference between the two methods of measurement.

It seems quite certain, however, that the borer does give higher results than the hatchet and rule. Since it has been shown above that even the bark borer underestimates bark thickness in red spruce, it may be assumed that the bark borer gives the more accurate estimate of actual diameter inside bark. That the borer should be superior from this point of view is also evident from the fact that the bearing of the borer on the bark is directly comparable to the bearing of the caliper arm or tape, and the danger of flaking off is very slight. On the other hand, if the object is to get a true picture of actual thickness of bark, without respect to diameter measurements, the hatchet and rule, when carefully used, may be preferable. It is surprising how strongly the reading of the bark borer is affected by projections and irregularities of no real significance in judging actual

³ Diameter breast high.

thickness of bark. Errors and abnormalities all tend to affect the reading in the same direction, so that there is no compensation whatever except the chance that the borer is not driven to the wood, and with careful manipulation there should be no danger of this. With the hatchet and rule, however, the bark is exposed in cross section for 2 or 3 inches and a reliable figure can be obtained if proper care is taken to avoid losing bark scales in chopping.

OFFICE WORK

PRELIMINARY CALCULATIONS

A taper curve of diameter inside bark and height above ground was plotted for each tree. Wherever the reverse curve of the root swell extended above breast height the normal convex curve of the middle portion of the stem was continued downward to breast height, and from this was read the "normal" diameter of the tree. Whenever the measurement at half height was clearly erratic or out of line with those above and below, it was modified by reading a new value from the smooth curve drawn through the other points. From the figures thus obtained the amount of butt swell at breast height, the normal form quotient, and the percentile tapers of each tree were computed.

For each separate locality or stand the average age, form quotient, form-point height, and average height of dominant and codominant trees were computed from the trees measured in that stand. The standard deviation of the form quotients of the trees measured in each stand was also calculated. For each stand the total number of trees per acre, the number of trees more than 3 inches in diameter, and the percentage of each species were calculated from the strip survey or sample-plot tally sheets.

CHECK ON FORM CURVE AND BUTT SWELL ALLOWANCE BY LOCALITIES

To aid in analyzing errors in volume tables, check the graphical elimination of butt swell and obtain further information upon the differences in average form curve in stands of different characters, a study was made of the fit of the material from each locality to the

formula $y = \frac{x}{a+bx}$, in which y is the ratio of the diameter at distance x from the tip to the normal diameter at breast height, the distance x being expressed as percentage of total height above breast height.

This formula when converted to the form $\frac{x}{y} = a+bx$, becomes the equation of a straight line.

For this purpose the percentile tapers of all the trees of each species in a locality were averaged and values of x/y were computed for the resulting average taper series. These values were then plotted x/y on x and conformity to a straight line noted.

The butt-swell eliminations were generally satisfactory, as shown by the breast-height point of the averages falling in line, and no good reason was found for modifying the graphs of the individual trees in the case of a few averages in which the results were not exact. There is, perhaps, a tendency to overestimate the butt swell in stands of very low form quotient.

The fit of the form curve in even-aged red spruce was good in general, but there was some tendency for the upper sections to fall below values given by the formula. The same applied to the old-growth red spruce, in which, however, the falling off in the tops was somewhat more common and more pronounced.

The even-aged balsam fir fitted very well in general, except in the swamp localities where the tops fell below the formula. In old-growth balsam fir the form curve varied systematically from the formula, the diameters being relatively larger below the mid-point and smaller above, resulting in a characteristic S curve for the plotting of x/y on x . Since the excess volume below the mid-point offsets the deficiency above, the effect on cubic volume is of no greater importance than in the cases of red spruce and even-aged balsam fir, which fitted better.

White spruce, on the whole, fits the formula better than either of the other two species. With this species the minor variations appear to be more or less definitely related to certain stand characters. Old-field stands fit very well when uniformly stocked. In extremely irregular stands the diameters in the tops do not hold up to the formula values, and in fully stocked natural second growth not of the old-field type the tops exceed the formula somewhat, except in the very young age classes.

The average taper series for each locality was then plotted x^2 on y and the corresponding absolute form factor obtained by planimetering the area under the curve. The differences between the actual absolute form factors thus obtained and the absolute form factors based on the formula corresponding to the average form quotients of the localities were then used to evaluate the effect of variation from the formula in terms of volume. Significance of errors was computed on the same basis as outlined in connection with table 6 of the writer's previous paper (2, p. 716). Any error was considered significant which exceeded the probable error in volume resulting from normal variation of upper diameters within a form-class range of five units as calculated for the number of trees in the sample.

In general the fit was good and the significant volume errors ran above 2 percent in only a few cases. A summary of the results is given in table 5.

TABLE 5.—Number of stand samples exhibiting significant volume errors of various magnitudes when measured by formula $y = \frac{x}{a+bx}$

Significant volume errors (percent) ¹	Even-aged			Old-growth		All stands
	Red spruce	White spruce	Balsam fir	Red spruce	Balsam fir	
	Number	Number	Number	Number	Number	Number
0.....	16	12	7	3	2	41
0.1-1.0.....	4	13	8	5	7	37
1.1-2.0.....	10	2	1	7	1	21
2.1-3.0.....	0	0	1	2	0	3
3.1-4.0.....	1	1	0	0	0	2
Total.....	31	28	17	17	11	104

¹ Errors in excess of probable error resulting from normal variation of upper diameters within a form-class range of 5 units.

CODING AND MACHINE TABULATION FOR MULTIPLE-CORRELATION STUDIES

To facilitate sorting and tabulating by the use of punch cards and Hollerith automatic machines, all the data to be used in multiple correlation calculations were coded. Species, localities, types, and crown classes were given arbitrary numbers to permit sorting on these factors, and the values for all the other variables were grouped so that the entire range would be included in from 10 to 30 code numbers.

The average height of dominant and codominant trees of red spruce at 50 years of age according to Meyer (8) was used as the basis for site classification of the various stands. Stands in which no red spruce occurred were converted to the red spruce standard by reducing heights of white spruce 7 percent and of balsam fir 9 percent, these factors also having been determined by Meyer in connection with his study of the yield of these species. For coding, the site indices were grouped into classes of 2 feet each.

Average heights of dominants and codominants were grouped into classes of 3 feet each. Numbers of trees per acre were coded in hundreds, and average ages were coded in groups of 5 years each. The standard deviations of form quotients in each stand were grouped into classes of 0.2 units each.

The crown lengths of the individual trees expressed as percentages of total height were coded in 10-percent groups. Form quotients were thrown into groups of 3 units each, and form points, calculated down from tip in percentage of total height, were grouped by 5 units each. Total heights were coded in 5-foot groups, and breast-high diameters in even inch classes. Double bark thickness, butt swell, and the sum of these two factors were coded directly in tenths of inches.

The procedure outlined by Smith⁴ for the solution of multiple correlation problems from punch-card tabulating-machine data was used throughout. In this work each species was kept separate, and old growth was kept separate from second growth. A list of all the data on all the cards was also run off on the machine to be used in checking errors in card punching, etc.

Smith's procedure for obtaining the coefficient for normal equations consists of the following steps:

1. Obtain mean of each variable from sum of all values and the number of observations.
2. Sort cards on first variable and tabulate the sums of all variables by this classification with count of numbers of observations in each class.
3. Multiply sum of each variable by class value of the sorted variable and add the products thus obtained. This gives the factors sum A^2 , sum AB , sum AC , etc., from the arbitrary origin 0.0.
4. Sort cards on second variable, and in same manner as for first sorting obtain the factors, sum AB , sum B^2 , sum BC , etc.
5. Repeat for all remaining variables.

⁴SMITH, B. B. THE USE OF PUNCHED CARD TABULATING EQUIPMENT IN MULTIPLE CORRELATION PROBLEMS. U. S. Dept. Agr. 24 pp. 1923. [Mimeographed.]

6. For each of the above sortings, multiply sum of sorted variables by means of all others. This gives products of the form $\sum x \cdot y_o$, which is equivalent to $N(x_o y_o)$ since $\frac{\sum x}{N}$. In this notation the subscript "o" denotes the mean.

7. Subtract products obtained in 6 from corresponding product sums obtained in 3, 4, and 5. This gives the product sums about the means of the variables as origins, and these are the coefficients needed for the solution of multiple-regression equations.

The procedure as given by Smith assumes that for any observation of one variable there will be corresponding observations of all the other variables under consideration. In assembling the data for this study there were a number of instances where observations on certain of the variables were not available. For this reason it became necessary to modify the calculation of the coefficients for factors involving these variables.

ADJUSTMENT OF CALCULATIONS FOR VARIABLES WITH INCOMPLETE DATA

Using a general notation in which x = a variable for which a smaller number of observations, N^1 , is available than in the case of y and other variables in the study, for which we have N observations, steps 3, 4, and 5 outlined above will give factors of the form $\sum xy$ composed of only N^1 products in all cases involving the short variable x .

For step 6 in the above outline with respect to variable x , determine first the true mean of this variable, which will be $\frac{\sum x}{N^1}$. Then obtain from the machine tabulation in which the sorting was done according to x the sums of the other variables for observations actually paired with observations of x . Multiply these sums by the actual mean of the variable x , which will give factors of the form $N^1(x_o y_o)$. These are to be subtracted from the products $\sum xy$ obtained in steps 3, 4, and 5. In order to use the coefficients thus obtained on the basis of N^1 observations in multiple correlation with other variables whose coefficients are based on N observations we must assume that the magnitude of the coefficients would be proportional to the number of observations. The coefficients for all factors involving the variable x must therefore be increased by the factor $\frac{N}{N^1}$. This assumption should not involve much danger so long as N^1 is not much smaller than N .

If more than one variable is incomplete, special care must be taken to use the proper figures in the calculation of the coefficient for the product of a pair of short variables. The correct number of cases in which the two short variables are actually paired must be ascertained, and this must be used to obtain, in conjunction with the sums of the variables in these cases, the true means of the variables, and also in making the final adjustment of the coefficient to place it on the basis of N observations.

In the present study this adjustment had to be made in the cases of crown length and form quotient based on the entire stem, but since the latter was not used in the final analyses, it affects only those relations in which crown length appears as a factor.

FACTORS OF AVERAGE FORM OF STANDING TIMBER

VARIABILITY OF FORM QUOTIENT

The present study substantiates the conclusions drawn in a previous paper (2) that a standard deviation of about ± 4.5 units will apply quite generally as an average representation of the variability of form quotients within a stand. The range of variability found among the stands measured was quite high, as might be expected with figures for each stand based on only 20 trees (table 6).

TABLE 6.—*Variability of form quotients in stands*

Type and species	Stands	Average of standard deviation of form quotients in stands	Range of standard deviation of form quotients in stand	Standard deviation of form quotients of individual trees from all stands
Even-aged stands:	<i>Number</i>			
Red spruce.....	31	4.71	3.0-6.7	7.52
White spruce.....	28	4.24	2.8-6.6	7.57
Balsam fir.....	17	3.92	2.3-6.0	5.39
Old-growth stands:				
Red spruce.....	16	4.58	3.2-5.6	5.22
Balsam fir.....	11	4.80	3.2-6.4	5.63

The variability of form quotients in stands does not seem to be associated very closely with any factor which can be readily evaluated. The variability tends to decrease as the number of trees per acre increases and as age increases. Variability is usually less in stands of high average form quotient than in stands of lower average form quotient. In the case of even-aged red spruce and balsam fir the number of trees per acre has more influence on variability of form quotients than has age, site, or average height, but for white spruce age is the most important factor.

DETERMINATION OF AVERAGE FORM BY FORM-POINT METHOD

In the application of the form-class volume-table system in Sweden the form-point method has been used for estimating the average form quotient of the various stands. The form-point method has been described in detail by Jonson (7) and outlined by Wright (9) and Behre (2). In his previous paper (2) the writer worked out in detail the application of the form-point relation for western yellow pine, now known as ponderosa pine, and preliminary functions for red and white spruce and balsam fir were prepared for use by Meyer (8) in connection with his yield tables for these species. Wright (9) gives rectilinear form-point relations for white spruce, black spruce, and balsam fir, but since in each case these are based on the data from only one locality, and since they take no account of curvature, they cannot have any general significance. It has been found that species differ in their relations between form point and form quotient, so that in this study separate relations were worked out for each of the five sets of data.

In previous work rectilinear relations have been used even though some curvature was indicated, but it now appears that the curvature is not only consistent in all species and usually of sufficient amount to affect materially the accuracy of estimate, but that it is associated with

definite growth conditions and, therefore, should be taken into account. The relation between form point and form quotient in general is such that the closer the form point lies to the tip of the tree, the higher the form quotient. When averages of a large number of trees are plotted, however, it will be found that very little if any increase in form quotient will accompany a movement of the form point closer to the tip than 25 percent of the total height. In fact, especially with white spruce, it will be found that form points extremely close to the tip are often associated with relatively low form quotients. In the field it will be observed that trees on which the form point as usually defined falls within 15 percent of the tip are commonly tall, slim, intermediate trees with thin-foliaged crowns of very limited development. These trees are generally closely sheltered by adjacent crowns, and the crown surface presented to the wind is so small in comparison with crowns of more normally developed trees and in proportion to their total area including bole that it ceases to be the dominant element in determining form quotient. That relatively low form quotients should be associated with the narrow crowns of these trees in spite of extremely high form points is in accord with the findings of Gevorkiantz and Hosley (4), which indicate that form quotient may be estimated accurately from crown length and width.

The form-point relations are presented in figures 1 and 2, and the accuracy of estimates of form quotients from these curves is shown in table 7.

TABLE 7.—Accuracy of estimates of form quotients from form point

Type and species	Standard deviation of form quotients	Accuracy of estimates of form quotient of single trees from form-point curves			Standard error of estimating average form quotient of stand from measurement of form point on 20 trees ¹	Number of trees required to attain standard error of average form quotient not over ± 1 unit ¹
		Standard error	Alienation index	Index of determination		
Even-aged stands:						
Red spruce.....	7.52	5.00	0.66	0.56	1.54	48
White spruce.....	7.57	5.04	.67	.55	1.47	44
Balsam fir.....	5.39	4.28	.79	.38	1.30	34
Old-growth stands:						
Red spruce.....	5.22	4.72	.90	.19	1.47	44
Balsam fir.....	5.63	4.52	.80	.36	1.47	44

$$^1 \text{ Based on formula } s_{Dax_1} = \sqrt{\frac{sDx^2 + sDd^2}{N}}$$

in which—

ax_1 = average of observed deviations.

x = true deviation (sDx from table 6, column 3).

d = error of observation (sDd from column 3. above).

N = number of observations.

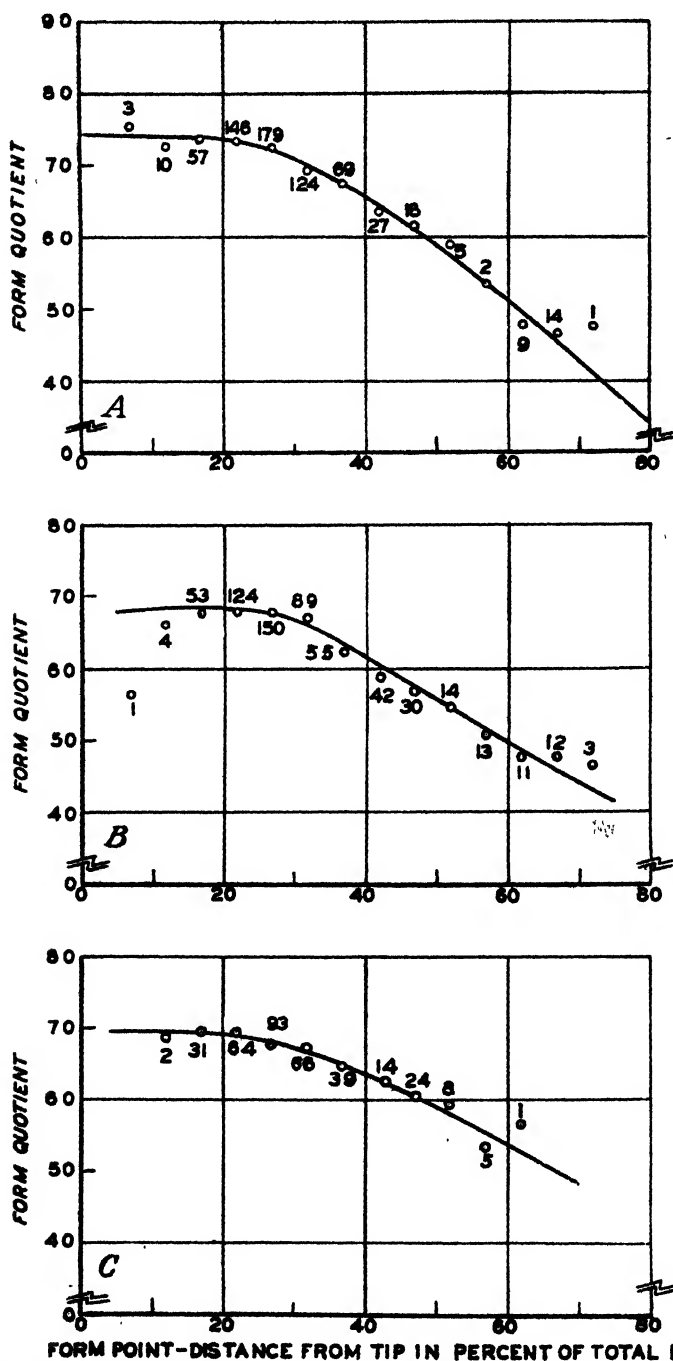


FIGURE 1.—Relation of form quotient to form point in even-aged stands: A, Red spruce; B, white spruce; C, balsam fir.

It will be seen from table 7 that although the standard errors⁵ of estimating the form quotients of individual trees from the form-point relations vary less than one unit between the five sets of data, yet there is a big difference in the alienation indices⁶ because of con-

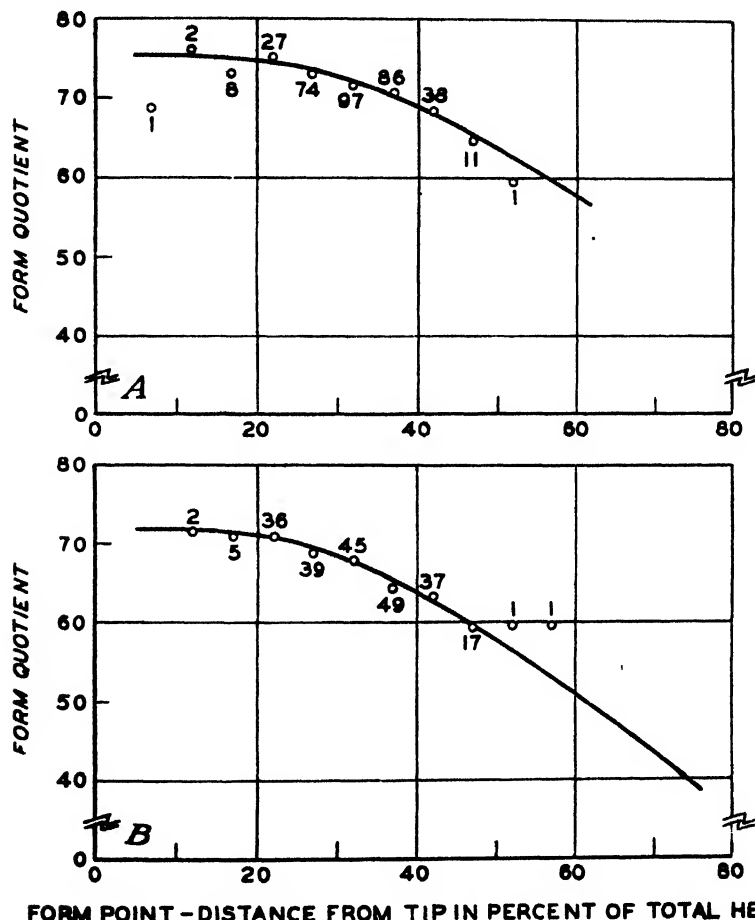


FIGURE 2.—Relation of form quotient to form point in old-growth stands: A, Red spruce; B, balsam fir.

siderable difference in the initial variability of the form quotients. Thus, although the use of form point reduced the variability of

⁵ The standard error of estimate is the standard deviation of the differences between the actual values of the dependent variable and the corresponding values as estimated from a curve, a regression equation, or an alignment chart. It measures the variation about the average relationship in absolute terms.

⁶ Alienation indices are used in this study as the primary measure of correlation. The alienation index is the ratio between the standard error of estimate of a dependent variable from any set of independent

variables and the original variation of the dependent variable; thus $AI = \frac{SE_{y,abc...}}{SD_y}$. When the relation-

ships are rectilinear the term "alienation coefficient" is used instead of alienation index. The alienation index is an abstract measure of the degree of relationship. Its chief merit is the fact that it gives a direct percentage measure of the improvement in estimating one factor from any functional relationship in which it may be involved. The index of determination is perhaps a better measure of the proportion of variation of the dependent variable which is associated with the independent factors which have been included in any problem. The index of determination is the square of the index of correlation and its relation to the alienation index is such that $CI^2 + AI^2 = 1$. In this paper indices of determination are given in each table along with alienation indices, in order to facilitate interpretation, no matter which measure the reader is accustomed to use.

estimate of individual form quotients about one-third in the case of even-aged red spruce and white spruce it fails to account for more than one-tenth of the error of estimate of form quotients in old-growth red spruce. On the other hand, it has been found (table 6) that the variability of form quotients in any single stand of old-growth red spruce will be about 4.6 units. The total variability of all the old-growth red spruce data was only 5.22 units, which, being so near 4.6, indicates that there could not have been any great difference in the average form of the various stands measured as compared to the even-aged stands, the data from which showed a total variability of 7.52 units.

With these species, just as with ponderosa pine (2), form point must be measured on approximately 45 trees in order that the standard error of the average form quotient be kept down to ± 1 unit.

DETERMINATION OF AVERAGE FORM FROM STAND AND TREE FACTORS

One of the principal objects of this investigation was to find out by statistical means whether form quotient was associated closely enough with any of the characters of the stand or of the trees so that it could be derived from measurements that are usually obtained during the course of a cruise or tally of a sample plot. This would eliminate the necessity for introducing a new conception like the form point, which requires special attention in the field and is not subject to exact determination even when well understood.

The characters of the stand that lend themselves to numerical evaluation for this purpose are site index, average height, number of trees per acre, and age. Average height of dominant and codominant trees and number of trees 3 inches and more in diameter were selected as the best measures for expressing height and density respectively. For purposes of studying form, basal area per acre is of little value as an indication of density because, at a given age, density and average form may vary widely without much change in total basal area. Basal area tends to remain constant because an increase in average size accompanies reduction in number of trees per acre. Number of trees 3 inches and more in diameter was found by statistical tests to be superior to total number of trees per acre for form studies, probably because it eliminates the comparatively large number of smaller trees in young stands that have ceased to exert any real influence on development of the future stand.

The measurable tree characters that may affect form quotient are diameter, height, length of crown, width of crown, and crown class. In planning this study it was thought that width of crown would be impractical to measure in the field, because of irregularity of crown outline, interlacing of adjacent crowns, and inaccessibility or invisibility of points of measurement, so no data were obtained on this factor. In the light of subsequent work in northern white pine by Gevorkiantz and Hosley (4) indicating the usefulness of crown width measurements in conjunction with crown or dead length, it is to be regretted that these measurements were omitted.

ANALYSIS OF TREE FACTORS

Correlation calculations involving various combinations of both stand and tree characters were used as a means of determining how each numerically measured factor is related to form quotient and

which of these factors have significant influence on the variation of form quotients among the individual trees. From multiple regression equations⁷ involving all the variables and affording some preliminary idea of the relative weight of each factor, it appears that diameter breast high, height, crown length, and site are the most important factors.

Multiple correlations involving form quotient and the four factors mentioned above were computed for the even-aged material and corrected for curvature by the method of Bruce and Reineke (3). The alienation indices and indices of determination are given in table 8. They represent the highest practical degree of accuracy that may be attained in the estimation of form quotient of individual trees. They mean that diameter breast high, height, crown length, and site together may account for 64 percent of the variability of individual form quotients in even-aged red spruce, for 66 percent in white spruce, and for 42 percent in even-aged balsam fir. More than a third of the total variability of form quotient is apparently associated with other factors and probably is not readily susceptible to numerical evaluation.

TABLE 8.—Accuracy of estimates of form quotients from individual tree factors

Type and species	Factors with which form quotient is correlated							
	Diameter breast high, height, crown percent, and site index		Crown percent		Diameter and height		Diameter, height, and crown percent	
	Aliena- tion index ¹	Index of deter- mina- tion ²	Aliena- tion index	Index of deter- mina- tion	Aliena- tion index	Index of deter- mina- tion	Aliena- tion index	Index of deter- mina- tion
Even-aged stands:								
Red spruce	0.60	0.64	0.69	0.53	0.73	0.46	0.64	0.59
White spruce58	.66	.62	.61	.64	.59	.58	.66
Balsam fir76	.42	.81	.35	.81	.34	.76	.42
Old-growth stands:								
Red spruce88	.23	.90	.20	.80	.36
Balsam fir75	.44	.88	.23	.75	.44

$$^1 \sqrt{1 - CI^2},$$

Study of the regression coefficients⁸ and curves of net regression⁹ in the several multiple correlations worked out show that diameter

⁷ Rectilinear functions fitted to the data by the method of least squares. Multiple regression equations express the function of a dependent variable in terms of any number of independent variables, without allowing for curvature in any of the individual relationships. They take the general form $y = a_1x_1 + a_2x_2 + a_3x_3 + \dots + a_Nx_N$.

$cx_2 + \dots + +N$. In a regression equation of the form $y = ax_1 + bx_2 + cx_3 + \dots + N$, the constants a, b, c , etc., are the regression coefficients of the several variables. The relative weight of each variable is indicated in a rough way by the size of these coefficients in relation to the absolute values of the different variables. Obviously the value of y in the regression equation will be more strongly influenced by a variable with a large regression coefficient than by one of about the same absolute value with a low regression coefficient. On the other hand, a low regression coefficient for a variable of large absolute values may have more weight than a large regression coefficient for a variable which never attains a high absolute value. But only a crude idea of relative weight may be obtained from the regression coefficients and absolute values because the rectilinear equation may distort badly the actual influence of any factor in respect to which the relationship is curved.

⁹ Net regression of any independent variable in multiple correlation is the relation between that variable and the dependent variable when the other independent variables are held constant at their means. Net regression curves are obtained by plotting the residuals of estimates of the dependent variables from the multiple regression equation around the original net regression line for each variable. The slope, degree of curvature, and closeness of fit of the net regression curves afford some indication of relative weight of the variables and may be used to supplement a study of the regression coefficients.

and height have substantially greater weight than the other factors but, having opposite signs, tend to compensate. This compensation is especially significant because there is a close association between diameter and height.

Examination of the deviations from the net regressions of form quotient on height indicate that form quotient increases rather rapidly with height until trees are about 40 feet tall, after which there is little further increase except in the case of white spruce, where it continues at a lower rate.

Data sorted by diameter classes show form quotients to be at a maximum at about 7 inches diameter in even-aged stands and at 8 or 9 inches in old growth. In the even-aged data the slope away from the maximum in either direction is relatively steep, each inch of diameter indicating a decrease of from 1 to 2.2 units of form quotient. In the old-growth data this decrease is much more gradual. The same tendencies appear in the plotting of deviation from the net regression of form quotient on diameter in multiple correlation with other variables.

This indication of a definite correlation between form quotient and diameter deserves further consideration, because previous work (2) had indicated that within stands the correlation between diameter and form quotient was so slight that an average form quotient could be safely used for the entire stand.

In order to study this point further, it was necessary to correlate deviations from mean form quotient with deviations from mean diameters within stands rather than to consider the association of form quotient with diameter breast high for the entire mass of material. Since the trees measured in each stand were arbitrarily selected to cover the range of sizes and crown classes exhibited rather than to give a weighted average for the stand, it was deemed advisable to use approximate median values as the basis for this calculation rather than the average of the trees that happened to be included in each stand sample. A composite sample of 5 to 10 stands taken at random from the available data was used for each species. In each case a significant but small negative correlation was obtained. The results are summarized in table 9. Although only 4 to 19 percent of the variation of the individual form quotients, as judged by the coefficients of determination, is associated with the size of the trees with reference to the medians for each stand, yet there will generally be a range of 6 to 15 units between the average form quotient of the smallest trees in a stand and the average of the largest. This is consistent with Wright's findings (9), although the differences he observes are exaggerated by the fact that root swell at breast height lowers the form quotients of the larger size classes abnormally in his calculations.

Similarly, if deviations from mean form quotient in each stand are averaged by crown classes there appears a definite tendency for the dominant trees to be lower in form quotient than either codominant or intermediate trees. Suppressed trees also average lower than intermediates, but not generally as low as the dominants. This tendency is most pronounced in red spruce where there is a range of between 2 and 3 units of form quotient between the dominant and codominant or intermediate trees in both old growth and second growth. It is of no significance in white spruce, where the range is only about 1 unit of form quotient.

TABLE 9.—Correlation of form quotient with diameter within stands

Type and species	Coefficient of correlation	Standard error of coefficient of correlation	Coefficient of determination	Allena-tion co-efficient	Regres-sion co-efficient	Approximate range of average form quotient by diameter classes	Basis	
							Stands	Trees
Even-aged stands:							Number	Number
Red spruce.....	-0.439	±0.057	0.193	0.598	-1.131	15	10	200
White spruce.....	-.377	±.061	.142	.926	-.825	9	10	201
Balsam fir.....	-.415	±.063	.172	.910	-1.124	6	5	100
Old-growth stands:								
Red spruce.....	-.420	±.082	.176	.908	-.668	12	5	100
Balsam fir.....	-.206	±.061	.042	.979	-.668	6	6	139

If the data are sorted by crown classes without respect to the individual stands, this tendency appears to be more pronounced, dominant trees averaging from 3 to 5 units lower in form quotient than intermediate trees, with the codominant class generally between these extremes. In no case, however, does consideration of crown class reduce the variation of form quotients more than 5 percent.

The variation of form quotients within stands is also associated with differences in the crown lengths of the individual trees. When deviations from median form quotients within stands are correlated with deviation from median crown percentages, it is found that the longer the relative length of crown the lower the form quotients (table 10). For red spruce and white spruce the trees with shortest crowns will average 7 to 8 units higher in form quotients than those with the longest crowns in the same stand. With even-aged balsam fir this relation has no significance, while in old-growth balsam fir it is much more definite than on either of the other species. This is interesting when considered in relation to table 9, in which old-growth balsam fir shows the least significant relation with diameter.

TABLE 10.—Correlation of form quotient and crown percent within stands

Types and species	Coefficient of correlation	Standard error of coefficient of correlation	Coefficient of determination	Allena-tion co-efficient	Regres-sion co-efficient	Approximate range of average form quotient by crown-percent classes	Basis	
							Stands	Trees
Even-aged stands:						Form quotient units	Number	Number
Red spruce.....	-0.367	±0.061	0.135	0.930	-0.161	7.5	10	200
White spruce.....	-.375	±.061	.141	.927	-.166	8.0	10	201
Balsam fir.....	-.110	±.099	.012	.994	-.045	1.5	5	100
Old-growth stands:								
Red spruce.....	-.302	±.090	.091	.953	-.134	7	5	102
Balsam fir.....	-.450	±.068	.203	.893	-.147	6	6	139

Crown percent is the only factor showing any appreciably crude correlation with form quotient when all the data for each species are thrown together; and indeed when the function is curved (figs. 3 and 4) the result is slightly better than when estimates of form quotient are based on diameter and height together (table 8), and about the same as that obtained by the use of form point (table 7). Wright (9)

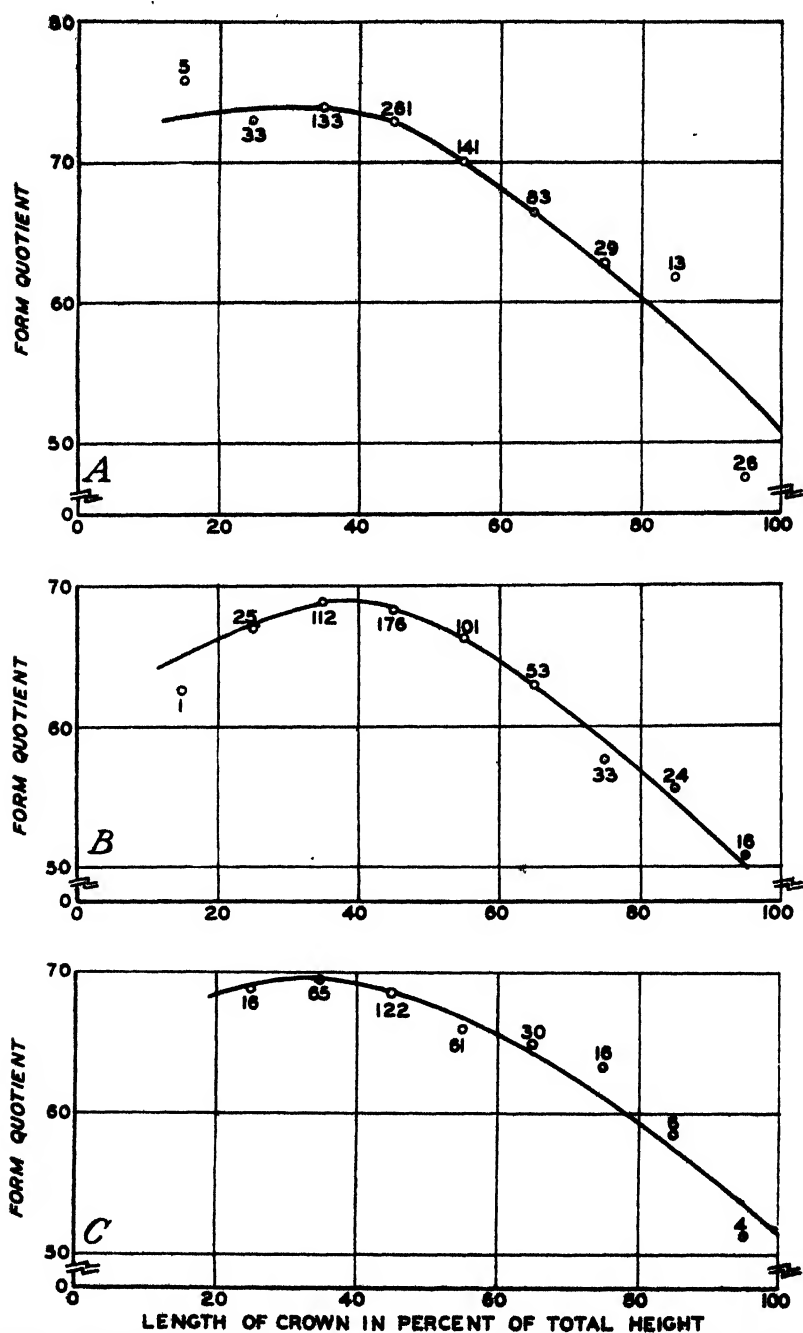


FIGURE 2.—Relation of form quotient of individual trees to crown length in even-aged stands: A, Red spruce; B, white spruce; C, balsam fir.

and Hedeby (5) also concluded that relative length of crown afforded as satisfactory a basis for estimating form quotient as the form point. Form quotients generally reach a maximum in trees with crown lengths about 40 percent of total height. With crowns longer than

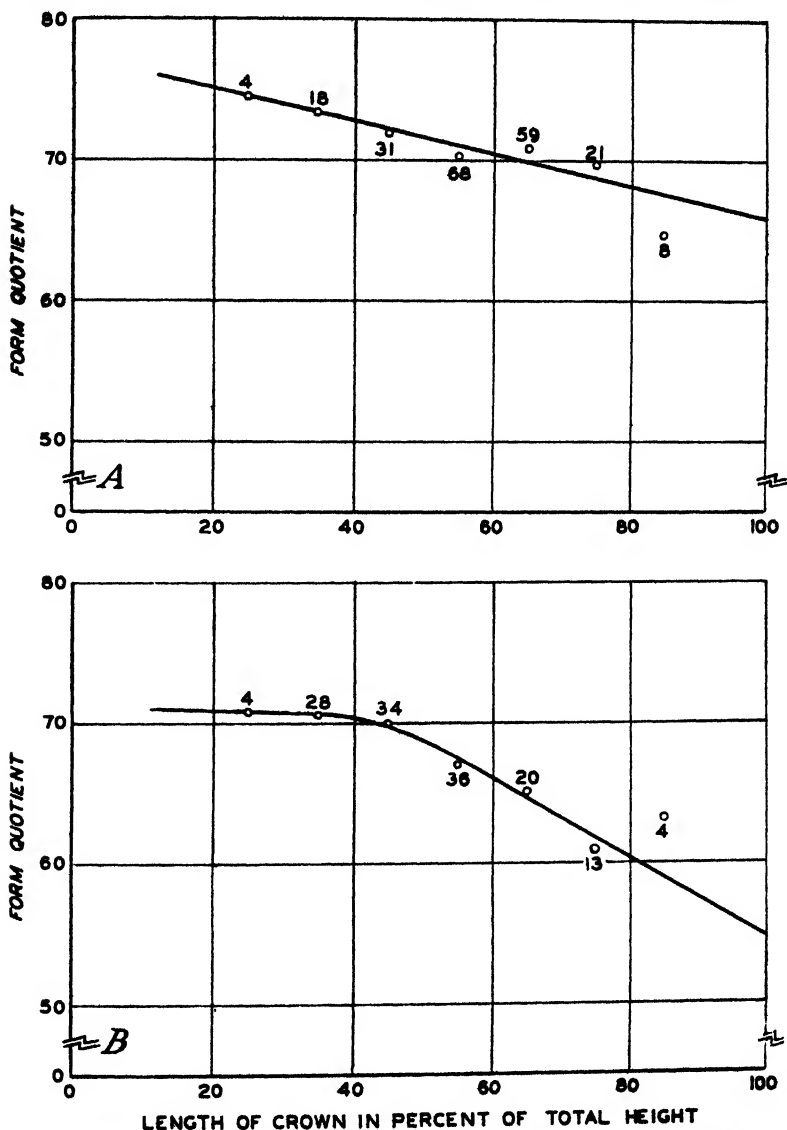


FIGURE 4.—Relation of form quotient of individual trees to crown length in old-growth stands. A, Red spruce; B, balsam fir

this there is a definite and steady decrease of form quotient and, just as was the case for trees with extremely high form points, form quotients average less than the maximum when crown length is less than 30 to 40 percent of total height.

From estimates on the basis of diameter, height, and crown percent, it will be seen (table 8) that, for white spruce and balsam fir, the results are just as good as when four factors were used, and that for red spruce they are within 4 percent of that accuracy. It would seem, therefore, that the fourth factor, site index, might be dropped as having little influence on form quotient beyond that which may be involved in the diameter-height-crown length relationship. What-

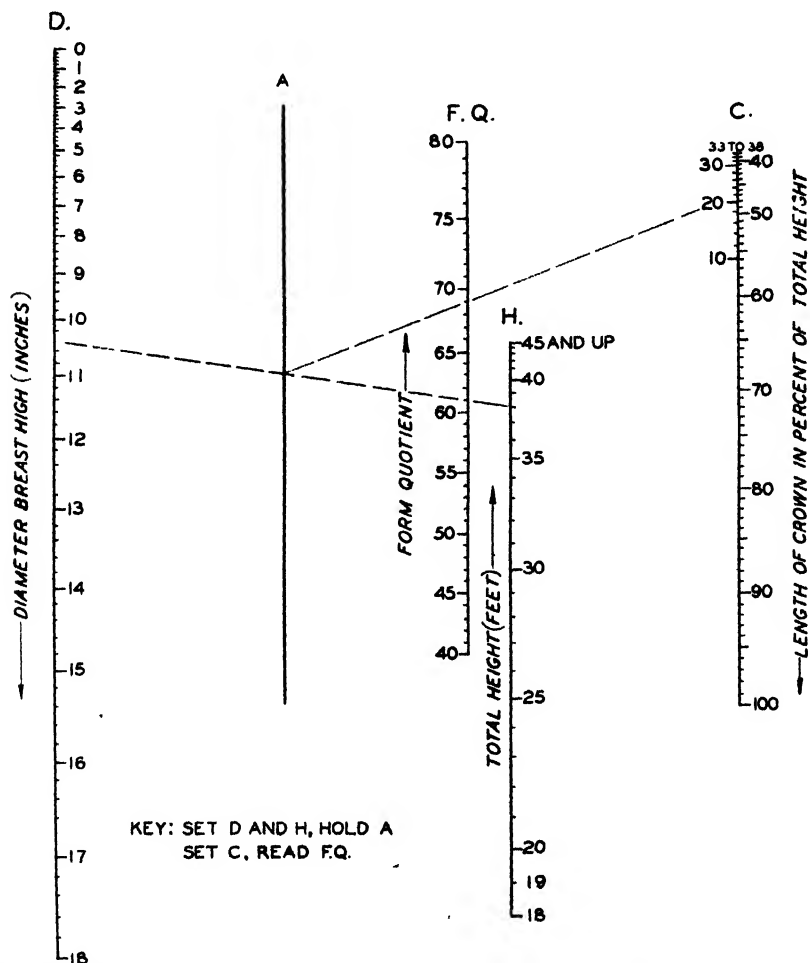


FIGURE 5.—Alinement chart for estimating form quotient of red spruce in even-aged stands from diameter, height, and crown length of individual trees

ever influence site index may have appears to cause trees on poorer sites to average higher in form quotient than do those on the better sites. That there is such a relationship is evidenced by the fact that estimates of form quotient based on diameter, height, and site index are almost as satisfactory as those based on diameter, height, and crown percent, especially in the case of even-aged red spruce, but it has not seemed possible to break down the interrelations between the

various factors to show definitely which elements may be the more fundamental.

Alignment charts for estimating form quotient from diameter, height, and crown percent are given in figures 5-9, and data indicating

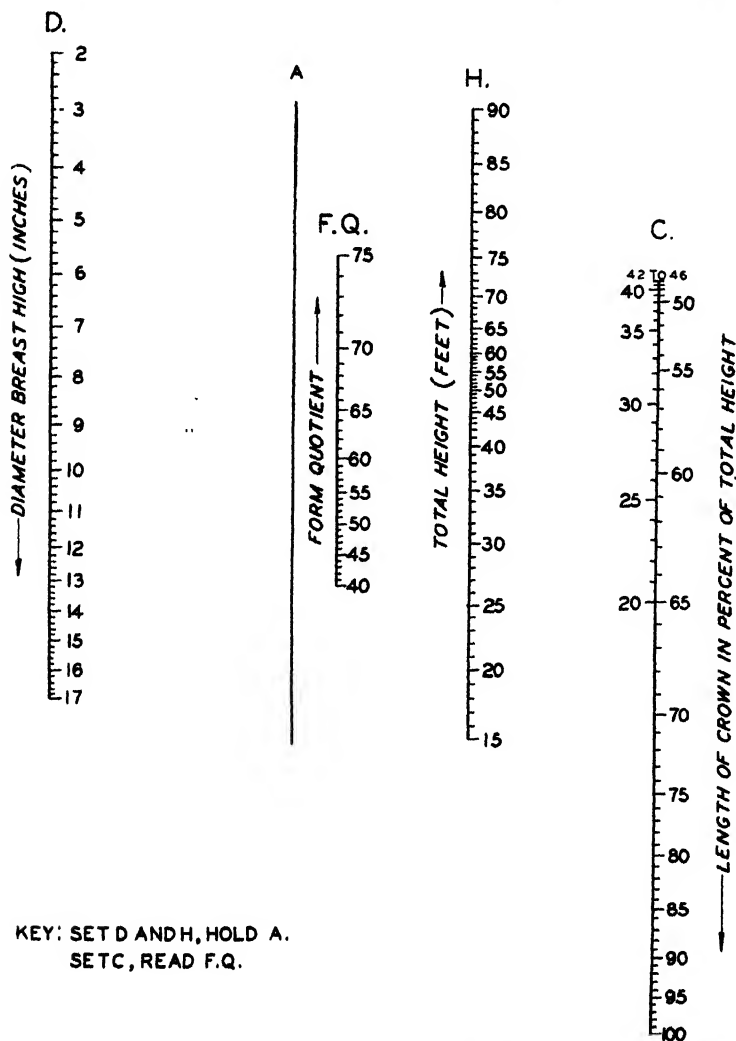


FIGURE 6.—Alignment chart for estimating form quotient of white spruce in even-aged stands from diameter height, and crown length of individual trees.

the accuracy of estimates from these charts are given in table 11. The results are somewhat better than those obtained by the form-point method (table 7) or from crown percent alone (table 8), but to obtain an average stand form quotient with standard error not over ± 1 unit still requires the measurement of about 40 trees.

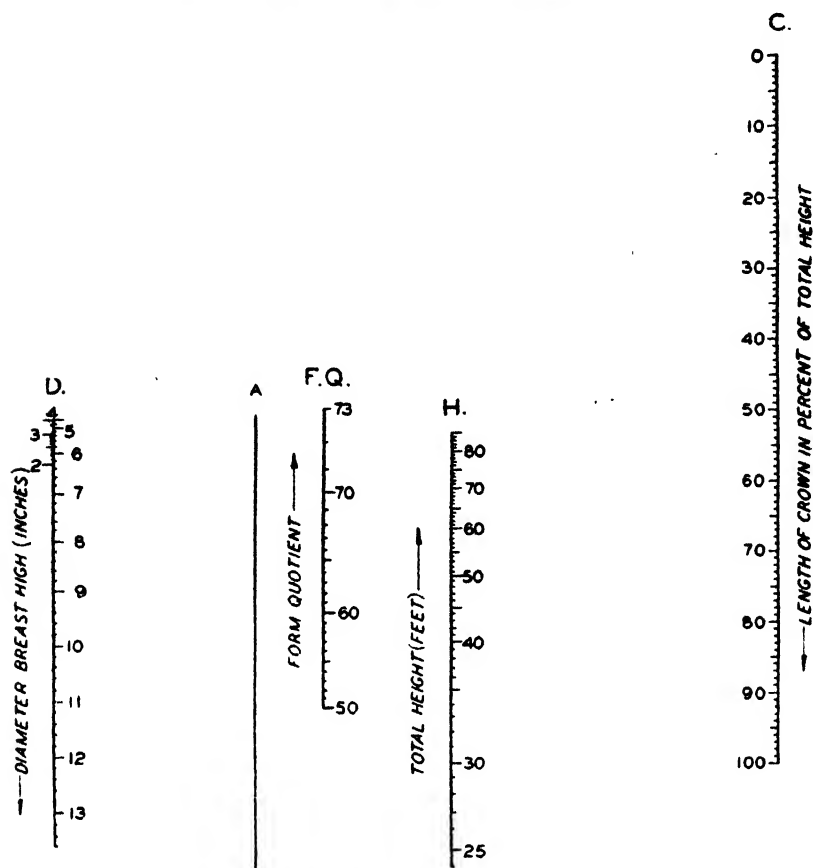


FIGURE 7.—Allinement chart for estimating form quotient of balsam fir in even-aged stands from diameter, height, and crown length of individual trees.

TABLE 11.—Accuracy of estimates of form quotient from diameter breast high, height, and crown percent

Type and species	Standard deviation of all form quotients	Accuracy of form quotient estimates of individual trees			Standard error of average form quotient of stand based on estimate of 20 trees ¹	Trees required to attain standard error of average form quotient not over ± 1 unit ¹
		Standard error	Allena-tion index	Index of deter-mination		
Even-aged stands:						Number
Red spruce.....	7.52	4.81	0.64	0.59	1.51	46
White spruce.....	7.57	4.39	.58	.66	1.36	38
Balsam fir.....	5.39	4.10	.76	.42	1.27	33
Old-growth stands:						
Red spruce.....	5.22	4.19	.80	.36	1.39	39
Balsam fir.....	5.63	4.24	.75	.44	1.43	42

¹ Based on formula $SD \sigma_1 = \sqrt{\frac{SD_1^2 + SD_2^2}{N-3}}$ in which—
 σ_1 = average of observed deviations.
 σ = true deviation (SD_1 from table 6, column 3).
 d = error of observation (SD_2 from column 3 above).
 N = number of observations.

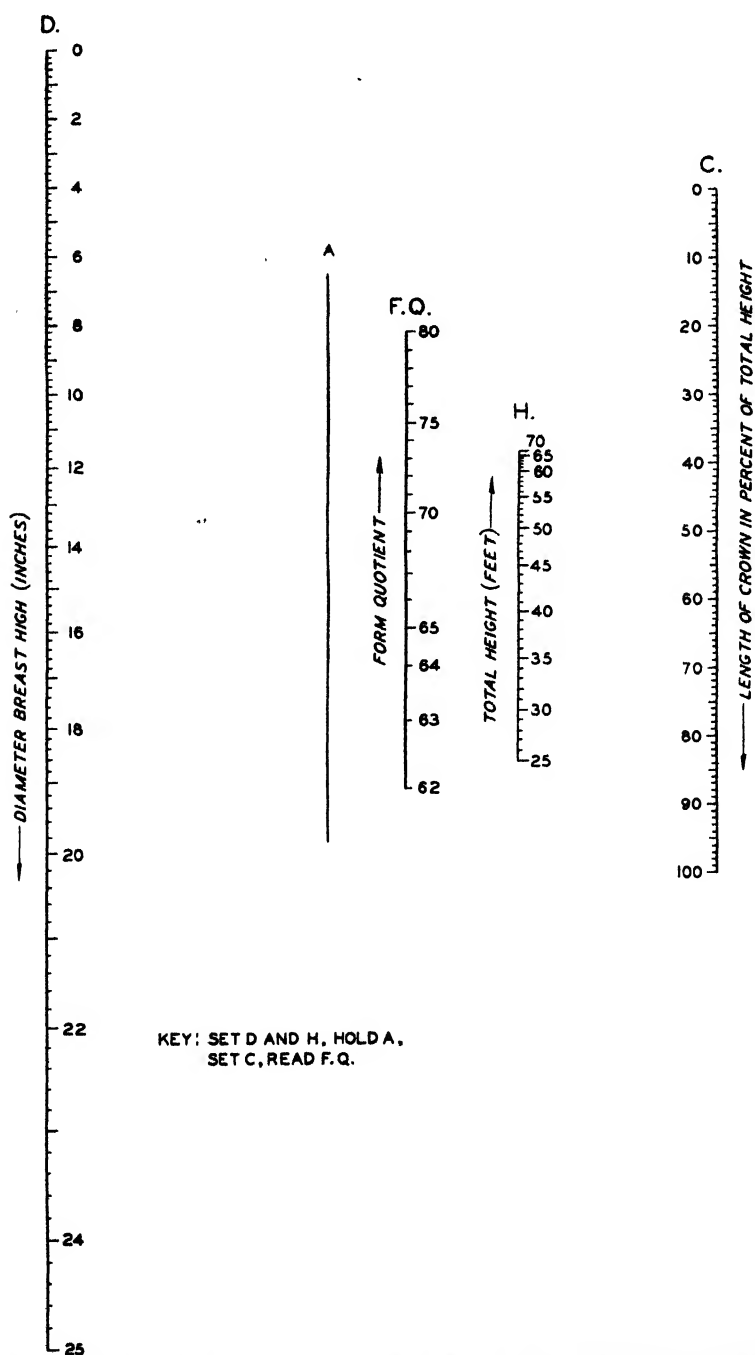
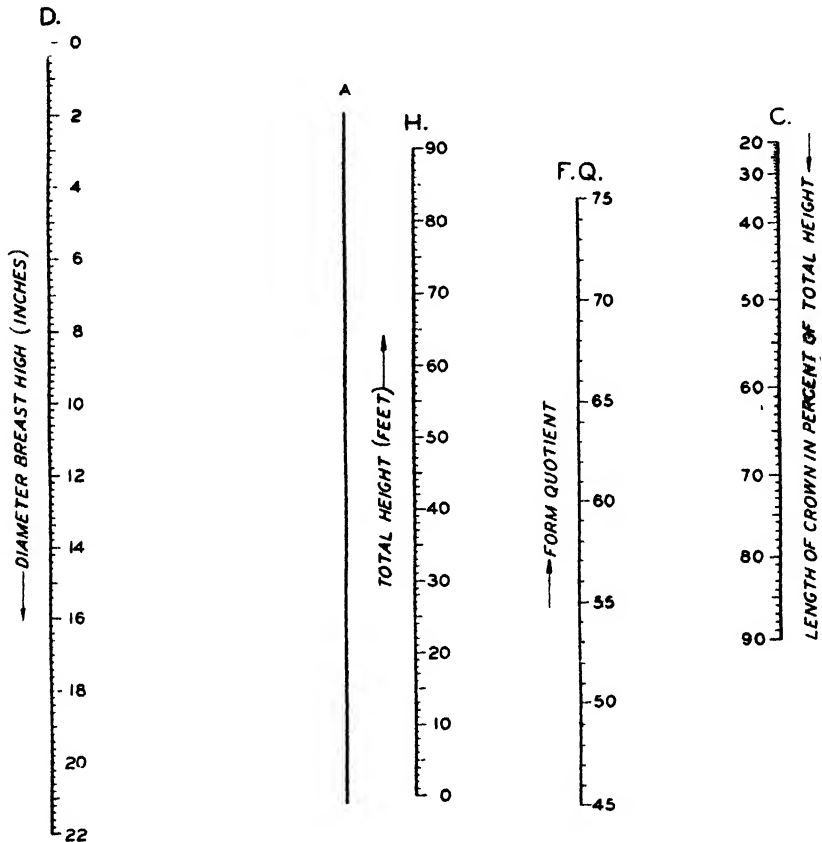


FIGURE 8.—Alignment chart for estimating form quotient of red spruce in old-growth stands from diameter, height, and crown length of individual trees.

To summarize these observations: It appears that variations in form quotient of individual trees are more closely dependent upon crown length than upon any other single factor and may be estimated on this basis just as well as by the form-point method. A more accurate estimate of form quotient may be obtained by considering diameter and height in conjunction with crown percent, and results



KEY: SET D AND H, HOLD A.
SET C, READ F.Q.

FIGURE 9.—Alinement chart for estimating form quotient of balsam fir in old-growth stands from diameter, height, and crown length of individual trees.

of about the same degree of accuracy may be obtained by using diameter, height, and site index.

Within the individual stands there is a slight but definite tendency for the larger trees or those with larger crowns to average lower in form quotient than the smaller ones or those with short crowns. Accuracy of estimates may, therefore, be slightly increased by subdividing the stand on the basis of size or crown length. But since this relationship is not very definite and since there is a compensation in that absolute form factors are not exactly proportional to form quotients (2, p. 731), it will seldom be necessary to apply this refinement in practice.

ANALYSIS OF STAND FACTORS

Since an average form quotient will generally be used for all the trees in each stand, it is desirable to investigate what association may exist between the characteristics of a stand and its average form quotient. Such a relationship may be more convenient to use than to estimate form quotient from a number of individual trees.

Because of the strong relationship already shown between crown percentage and form quotient, it is natural to suppose that the average form quotient of the stand would be closely associated with its relative crown length, which might well be measured as the average crown percent of the dominant and codominant trees. From table 12 it will be seen that 56 to 60 percent of the standard deviation and 81 to 84 percent of the variance of average form quotients of even-aged stands is associated with average crown percentages. The residual variation does not seem to be associated with either age, number of trees per acre, site index, height, or average diameter. The number of old-growth stands for which data are available is too small to yield any statistical expression of results, but for old-growth balsam fir only 1 of the 6 available stands varies more than 2 units of form quotient from a straight line fitted to the data. For old-growth red spruce, however, the points are more scattered although a similar trend is indicated.

TABLE 12.—Accuracy of estimate of average form quotient of even-aged stands from average crown percent and from age and number of trees per acre more than 3 inches in diameter

Species and number of stands	Standard deviation of average form quotients	Accuracy of estimates of average form quotient from average crown percent			Accuracy of estimates of average form quotient from age and number of trees per acre		
		Standard error	Alienation index	Index of determination	Standard error	Alienation index	Index of determination
Red spruce, 31.....	6.32	2.51	0.40	0.84	2.85	0.45	0.80
White spruce, 27.....	6.60	2.75	.42	.82	3.53	.53	.72
Balsam fir, 15.....	3.92	1.71	.44	.81	1.62	.41	.83

Further analysis of the even-aged material by Bean's method of graphical multiple correlation (1) shows that the average form quotient is also definitely correlated with age and number of trees per acre. Age is the more important factor, especially in earlier years of life. Up to about 70 years of age the average form quotient in even-aged stands of spruce and fir is increasing rapidly. After that time red spruce and balsam fir show little, if any, change in form, whereas in white spruce form quotient continues to rise but at a slower rate. Wright (9) states that form quotient increases with age up to about 50 years. The average form quotient also increases as the number of trees per acre of more than 3 inches in diameter increases, up to about 1,200. Beyond this density no increase is noted; in fact, in the case of balsam fir there is a slight tendency for stands with more than 1,200 trees per acre to have somewhat lower form quotients. Wright (9) also observed an increase of form quotient with density (basal area) within certain limits. Little improvement in accuracy is attained when average height or site index are taken into consideration.

Alinement charts for estimating average form quotient from age and number of trees per acre of more than 3 inches in diameter are given in figures 10, 11, and 12.

That variations in age and number of trees per acre reflect approximately the same condition as variations in average crown percent is evidenced by the fact that residuals from form quotients estimated from crown percent do not appear to be correlated at all with either age or number of trees per acre, and that estimates of form quotient from age and number of trees per acre can be only slightly improved by consideration of average crown percent.

Although these measures appear less accurate (table 12) than those based on characteristics of individual trees (compare standard errors of estimate with those in table 7 and table 11), the indices of determination are fairly high and it is probable that they will be just as satisfactory in practice.

For the many-aged and mixed stands of old growth characteristic of the spruce-fir forests, average crown percent is the only one of these factors having any significance, and, as already mentioned, the relation between form quotient and crown percent is not clear-cut in old-growth red spruce. However, the old-growth stands included in this study, as already pointed out (p. 682), show a relatively restricted range of variation in average form, and study of the characteristics of the individual stands reveals a fairly distinct grouping which should make it possible to assign each stand encountered in the field to its proper form class by inspection and without any measurements. Thus, old-growth red spruce falls into two groups as follows:

F. Q. 73 applies to old-growth red spruce in closed stands quite generally, whether pure or in varying mixtures with hardwoods. Swamp types with long narrow crowns and drooping branch habit (black spruce) also fall into this group.

F. Q. 70 applies to heavily culled or broken stands of spruce and hardwoods covering a wide range of sites and mixtures.

With old-growth balsam fir the distinction indicated for red spruce does not seem to hold, but all the stands group themselves around F. Q. 67, which may be taken as a general average, or average crown percent may be used to get a closer estimate.

This association of fairly constant average form quotient with easily recognized stand characteristics applicable over wide areas, is in accordance with previous observations of the writer (2) for ponderosa pine and of Wright (9) for northern white pine and black spruce. It should be noted that since Wright made no allowance for butt swell in the calculation of individual form quotients, it is not inconsistent that he should assign slow-growing black spruce to F. Q. 68, whereas the writer, having corrected for butt swell, places these stands in the F. Q. 73 group.

COMPARATIVE FORM OF SPECIES

In studying the conditions in the wide variety of stands included in this investigation, it soon became apparent that there were certain significant differences in form between the three species studied under identical stand conditions. The differences in question are not those arising from slight variations in type of stem form referred to under Check on Form Curve and Butt Swell Allowance by Localities (p. 675), but rather represent differences in form quotient or relative

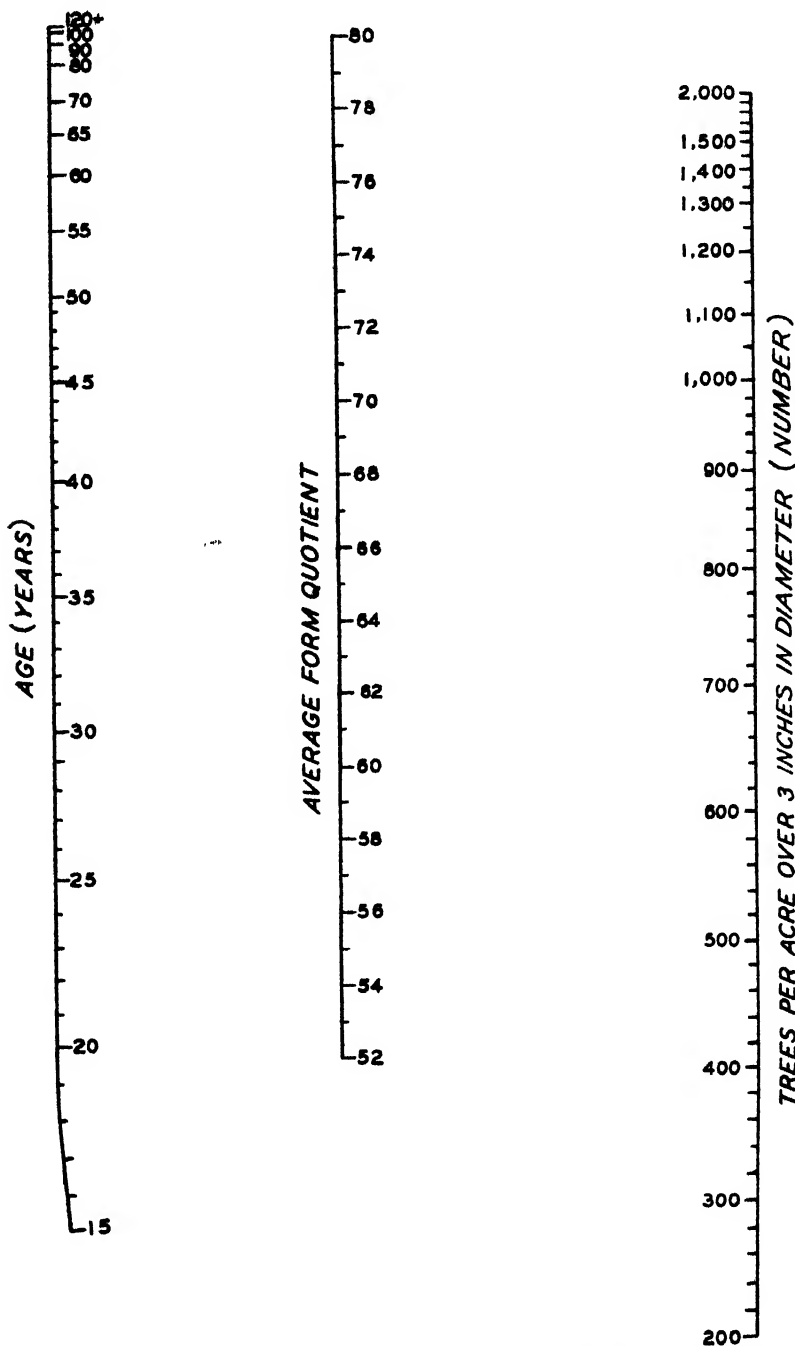


FIGURE 10.—Alignment chart for estimating average form quotient of even-aged stands of red spruce from age of stand and number of trees per acre over 3 inches d. b. h.

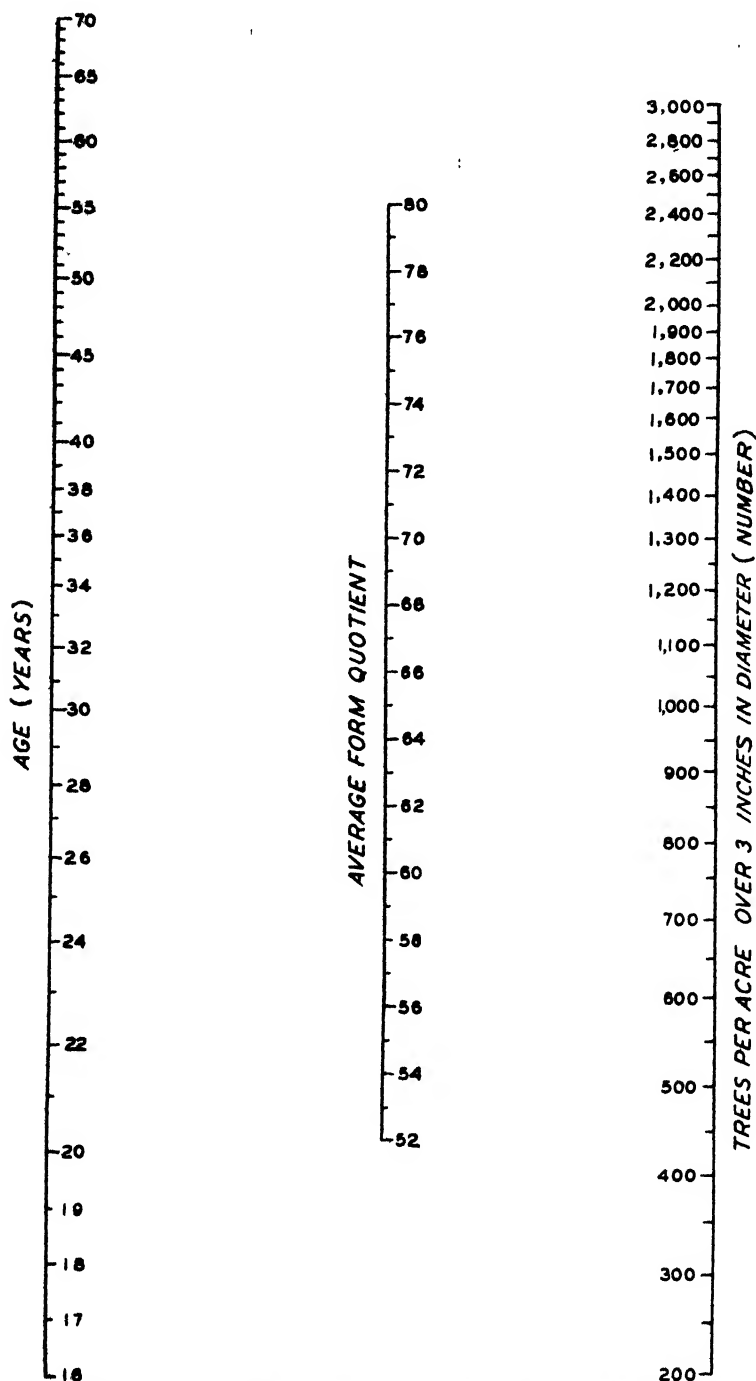


FIGURE 11.—Alignment chart for estimating average form quotient of even-aged stands of white spruce from age of stand and number of trees per acre over 3 inches d. b. h.

taper with the basic stem form essentially similar. Such differences are best illustrated by a comparison of the average form quotients in mixed stands.

Red spruce and balsam fir occur together quite generally in mixture with hardwoods in the old-growth stands of the region. Red spruce and balsam fir are also found quite frequently together in even-aged second growth. White spruce is absent from old-growth stands and occurs almost entirely in the even-aged old-field stands of second

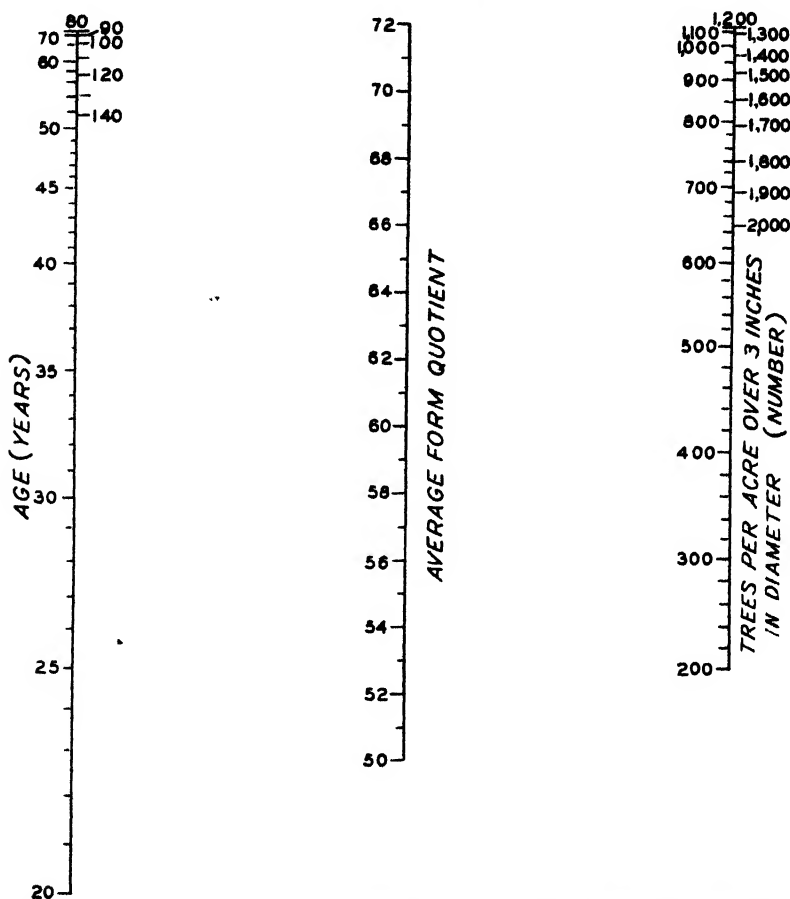


FIGURE 12.—Alignment chart for estimating average form quotient of even-aged stands of balsam fir from age of stand and number of trees per acre over 3 inches d. b. h.

growth. It is most commonly found in pure stands, but when in mixture is more frequently accompanied by balsam fir than red spruce.

Meyer (8) observed that red spruce would average considerably shorter in height than either balsam fir or white spruce in even-aged mixtures. When average form quotients are considered it is found that red spruce runs about 4 units of form quotient higher than either white spruce or balsam fir. The difference between red spruce and balsam fir is maintained in old growth as well as in even-aged second

growth, although both species will average higher in form quotient in old growth. Balsam fir and white spruce do not differ appreciably in form quotient when occurring together.

These differences are substantiated by examination of the charts showing the relations between form quotient and form point, crown percent, or diameter, height, and crown percent together. For the range of form points, crown lengths, and sizes most commonly occurring, the red spruce charts will indicate form quotients higher than either balsam fir or white spruce. In the case of charts based on the stand factors (age and number of trees per acre) no appreciable difference is apparent between red spruce and white spruce, although values for balsam fir run lower and appear consistent with the other tests cited.

FACTORS OF BUTT SWELL AND BARK THICKNESS

The accuracy of estimate of standing timber from volume tables is influenced by variations in bark thickness and butt swell of the timber in question as well as by variations in average form quotient. It is important, therefore, to attempt to find out what factors are related to variations in bark thickness and butt swell. This is especially important where basic form-class tables, such as those advocated by the writer, presuppose the elimination of butt swell from the "normal" diameter at breast height. For the use of such tables some basis is needed for reducing actual diameter breast high measurements to normal diameters. But even when the basic taper tables do not assume the elimination of butt swell, it is necessary to reduce outside bark measurements to inside bark diameters, and, since it should prove feasible to combine allowance for butt swell with the bark-thickness reduction, no extra work is involved in the first instance.

In this study an attempt was made to analyze bark thickness and butt swell at breast height independently. Coefficients of multiple correlation were worked out for each of these with diameter, height, form quotient, crown length, crown class, age, number of trees per acre, and site index.

BUTT SWELL

In the case of butt swell only a small proportion of the total variation can be accounted for by these numerical factors, and practically all the variation so accounted for is associated with diameter, the larger trees, of course, having the most butt swell at breast height. Variations in butt swell are much more closely correlated with size in old-growth stands than in second-growth stands, but this might be expected from the fact that the second-growth data were intentionally gathered from stands covering as wide a range of conditions as possible and were, therefore, much more heterogeneous than the old-growth data. Consideration of crown percent or form quotient may slightly improve estimates of butt swell from diameter alone, but this is not clear-cut or consistent in all cases.

The rectilinear alienation coefficients and coefficients of determination brought together in table 13 illustrate the various relationships. Only in the case of old-growth red spruce has it been possible to account for more than 18 percent of the standard deviation of butt swell. Although these coefficients do not represent the full effect of correlation between diameter and butt swell, because definite curva-

ture is present in the case of even-aged white spruce, old-growth red spruce, and old-growth balsam fir, still it is apparent that most of the variance is associated with differences in hereditary characteristics, or with differences in individual situation or anchorage which are not susceptible of numerical evaluation.

TABLE 13.—Correlation of butt swell at breast height with various factors

Factors with which butt swell is correlated	Even-aged stands						Old-growth stands			
	Red spruce		White spruce		Balsam fir		Red spruce		Balsam fir	
	Aliena-tion co-efficient	Coeffi-cient of deter-mina-tion	Aliena-tion co-efficient	Coeffi-cient of deter-mina-tion	Aliena-tion co-efficient	Coeffi-cient of deter-mina-tion	Aliena-tion co-efficient	Coeffi-cient of deter-mina-tion	Aliena-tion co-efficient	Coeffi-cient of deter-mina-tion
All numerical factors.....	0.85	0.28	0.86	0.26	0.90	0.18	0.63	0.60	0.82	0.33
Diameter breast high alone.....	.88	.22	.92	.15	.95	.09	.67	.55	.85	.28
Diameter breast high and crown percent.....	.88	.22	.91	.18	.94	.12	.66	.56	.84	.29
Diameter breast high and form quotient.....	.88	.23	.92	.15	.94	.12	.67	.55	.83	.31

The amount of butt swell in relation to size of trees is shown in figures 13 and 14. It is about the same in even-aged red spruce and white spruce. The diameter at breast height becomes affected slightly in the 3-inch or 4-inch class, but the amount of butt swell does not exceed 0.2 inch on the average until the trees get beyond the 12-inch class. The amount of butt swell appears to increase sharply for the larger trees, but the data are not strong enough to fix its trend very definitely. In even-aged balsam fir the amount of butt swell in relation to size of trees is only about half of what it is for red and white spruce, and in fact may be almost disregarded as it will seldom exceed 0.1 inch as an average for any of the size classes attained by this species.

The butt swell of old-growth timber is substantially greater than that of even-aged stands for both red spruce and balsam fir. In both cases, starting with trees about 5 inches in diameter, the rate of increase is at first gradual, but becomes quite rapid for trees above 12 inches in diameter. For this size class, butt swell will average about 0.4 inch for red spruce and 0.3 inch for balsam fir. For the 24-inch class the breast-high diameter of old-growth red spruce is enlarged about 2 inches by butt swell.

Some additional points on the behavior of butt swell were obtained by a detailed check of preliminary form-class volume tables prepared from data used in this study. The percentage of total tree volume represented by butt swell as determined by the technic used¹⁰ appears to be fairly constant in trees of all sizes in the case of even-aged white

¹⁰ The difference between total tree volumes, including butt swell, and "normal" volumes was obtained by planimetric charts of cross-sectional areas plotted over height above ground. For the normal volumes, the diameters used were obtained by prolonging the normal convex curve of the main portion of the stem to the ground line as described in Preliminary Calculations (p. 674). For the full volume including butt swell it was necessary to prolong the butt swell by eye down to the 1-foot point, because no measurements had been taken below 2.5 feet aboveground. The stump was considered a cylinder below the 1-foot point.

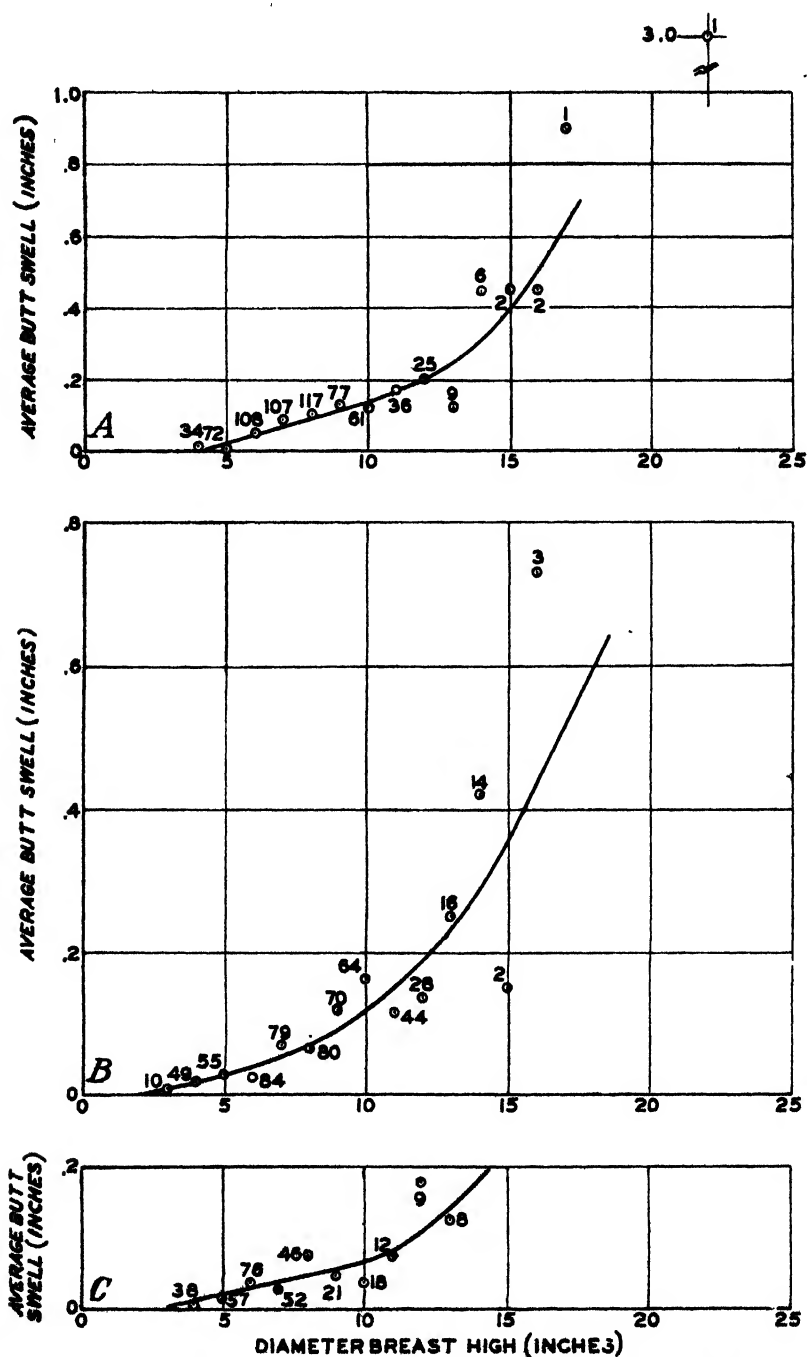


FIGURE 13.—Relation between butt swell and diameter at breast height in even-aged stands: A, Red spruce; B, white spruce; C, balsam fir.

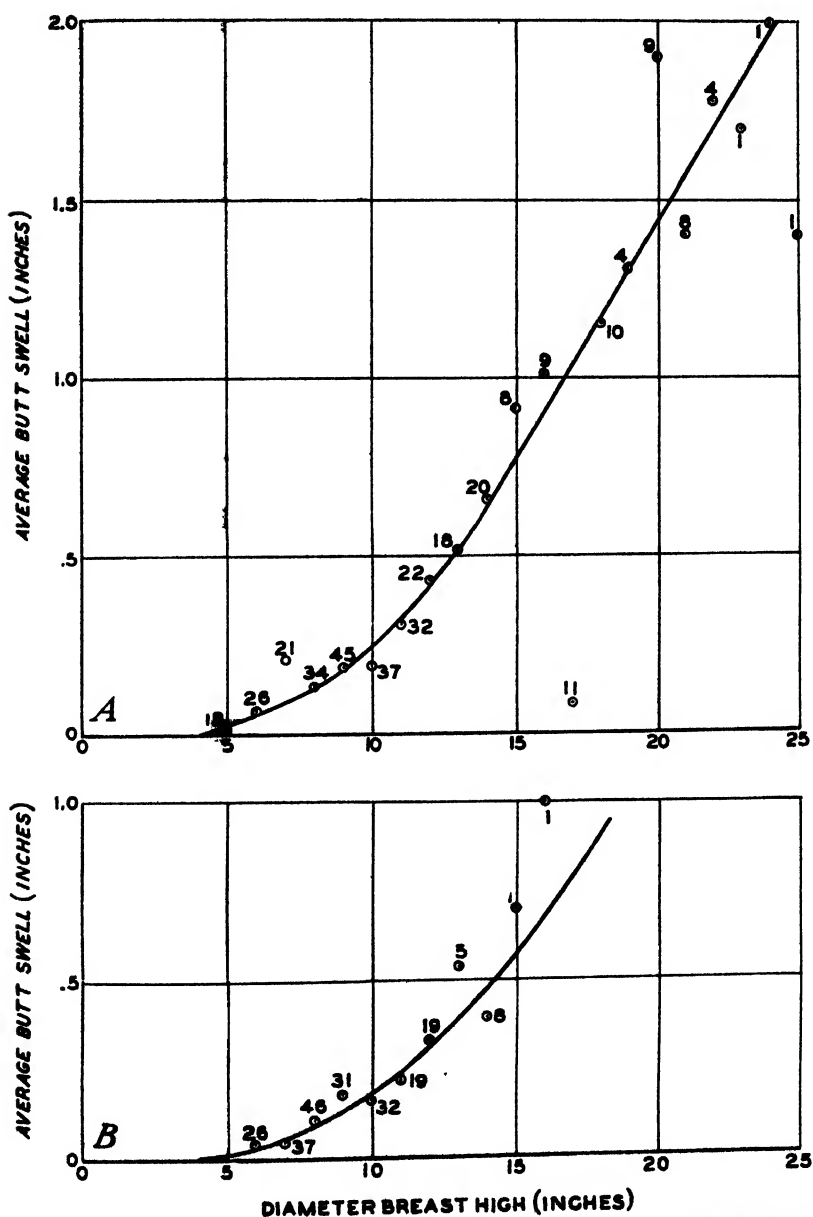


FIGURE 14.—Relation between butt swell and diameter at breast height in old-growth stands: Red *A*, spruce; *B*, balsam fir.

spruce and balsam fir, averaging about 2 percent for the former and 1½ percent for the latter. In the case of even-aged red spruce, however, it increases from 0 for 2-inch trees to 5 percent for 16-inch trees. When analyzed by localities it appears that the percentage of normal volume represented by butt swell decreases as average form quotient of the stand increases, and increases slightly with age.

BARK THICKNESS

When bark thickness is analyzed in similar fashion it is found that a much larger proportion of the total variation is associated with the numerical factors than was the case with butt swell. In the old-growth stands and in the case of even-aged red spruce practically all the variation which can be accounted for is associated with diameter, but with even-aged white spruce and balsam fir there is a substantial margin associated with other measurable factors.

By considering age or site index along with diameter a close approach to the total possible reduction of variation is made in all cases, but the effect of these factors does not appear to be consistent. In the case of white spruce, site index is the most effective factor after diameter, while age has very little influence. On the other hand, in the case of even-aged balsam fir, age accounts for nearly all the possible reduction of variation not associated with diameter.

To indicate the various relationships, a number of rectilinear alienation coefficients are presented in table 14.

TABLE 14.—*Correlation of bark thickness at breast height with various factors*

Factors with which bark thickness is correlated	Even-aged stands						Old-growth stands			
	Red spruce		White spruce		Balsam fir		Red spruce		Balsam fir	
	Alienation coefficient	Coefficient of determination	Alienation coefficient	Coefficient of determination	Alienation coefficient	Coefficient of determination	Alienation coefficient	Coefficient of determination	Alienation coefficient	Coefficient of determination
All numerical factors.....	0.55	0.70	0.50	0.75	0.54	0.71	0.51	0.74	0.63	0.60
Diameter breast high alone.....	.58	.66	.62	.62	.76	.42	.55	.70	.66	.56
Diameter breast high and age.....	.57	.68	.62	.62	.57	.67	-----	-----	-----	-----
Diameter breast high and site index.....	.56	.68	.56	.69	.68	.53	.55	.70	.66	.56

The net effect of age on bark thickness is positive, i. e., for trees of a given diameter class the older the tree the thicker the bark. The net effect of site index is generally negative, i. e., other things being equal, trees on better sites will have thinner bark than those on poor sites.

The bark thickness at breast height of even-aged red spruce appears to bear a constant relationship to size, averaging 7 percent of the outside bark diameter. For old-growth red spruce, bark thickness in relation to diameter decreases with increasing size of trees. Up to about 10 inches it is approximately the same as in even-aged stands, tending, however, to be slightly higher than 7 percent for the smaller trees; but it falls off in the larger sizes so that it is only 5.3 percent in

the 24-inch class. This is probably the result of a sloughing off of the scaly outer bark from the old trees more rapidly than new layers are added from within.

In balsam fir the bark thickness in relation to diameter is greater for all sizes in the case of old-growth timber than for even-aged second growth. Unlike that for red spruce, the curve for old-growth balsam fir maintains a constant relation to diameter, while the curve for even-aged second growth falls off slightly with increase in size. This situation is probably due to the difference in bark character of the two species. The smooth bark of balsam fir does not slough off, so a constant relation to size is maintained in the older stands. For old-growth balsam fir, the bark thickness at breast height is maintained at about 5.8 percent of the outside bark diameter for all sizes, while in even-aged stands it drops to about 4 percent for 12-inch trees.

The bark thickness of white spruce is about the same as that of red spruce in the very small size classes, but it becomes relatively less between 5 and 10 inches in diameter and maintains a lower ratio in the larger sizes. It constitutes about 6.3 percent of the diameter breast high in the 6-inch class and 5.2 percent in the 12-inch class.

These relations are illustrated in figures 15 and 16, in which it will be seen that white spruce is about midway between balsam fir and red spruce in respect to bark thickness. By comparison with figures 13 and 14 it is also evident that the amount of butt swell affecting breast-high diameter is never more than half the double bark thickness in trees of these species less than 12 inches in diameter. Butt swell becomes more important than bark thickness only in the case of old-growth red spruce larger than 17 inches d. b. h.

TOTAL REDUCTION OF DIAMETER BREAST HIGH FOR BARK THICKNESS AND BUTT SWELL

Study of each of the factors affecting butt swell and bark thickness having shown that variation in each factor is chiefly associated with variation in diameter breast high, it should be feasible to provide a basis for making allowance for both of these elements together. This should be the most practical procedure, since it is the sum of bark thickness and butt swell at breast height which affects the accuracy of the application of any volume table rather than either of these elements alone.

The relation of total reduction for butt swell and bark thickness to diameter at breast height is shown in figures 17 and 18. It will be seen that there is less curvature than was the case in the plotting of each element separately, because the curvatures of butt swell and bark thickness are in opposite directions and therefore tend to compensate.

The standard errors of estimate from these curves and the corresponding alienation indices and indices of determination are shown in table 15. It appears that the total reduction of breast-high diameter can be estimated from diameter breast high alone, with a standard error not exceeding 0.2 inch in all cases except that of old-growth red spruce, yet the correlation is considerably closer in the case of old-growth red spruce than in any of the other sets of data. Conversely the closest estimate is obtained in the case of even-aged balsam fir where the correlation is lowest.

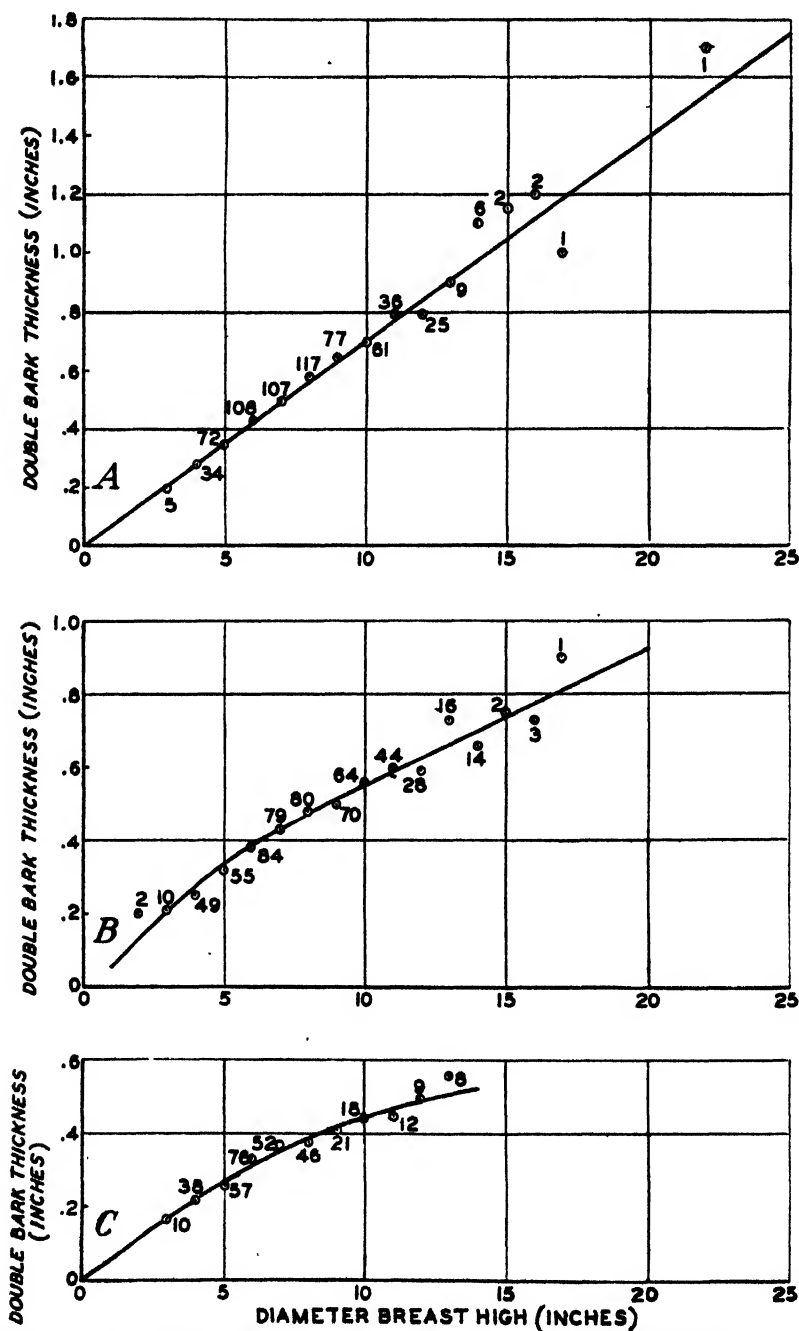


FIGURE 15.—Relation between bark thickness and diameter at breast height in even-aged stands: A, Red spruce; B, white spruce; C, balsam fir.

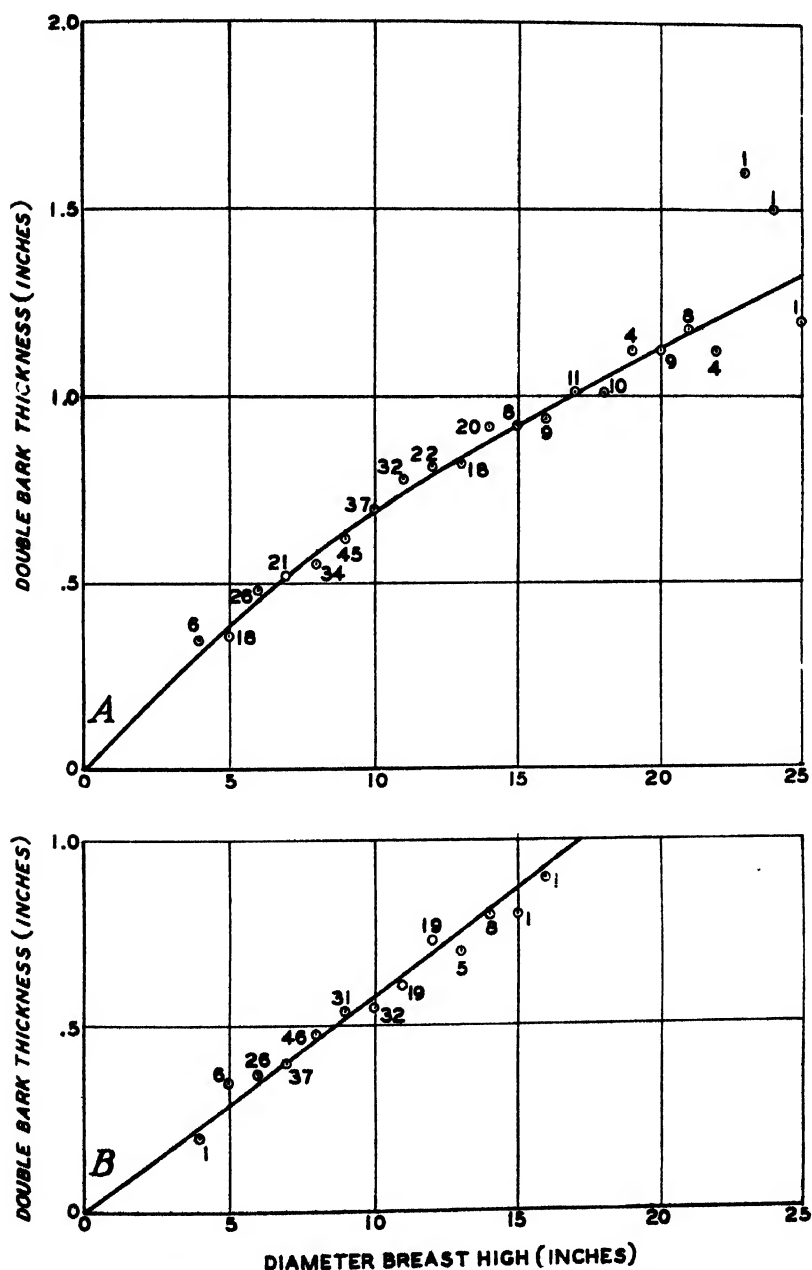


FIGURE 16.—Relation between bark thickness and diameter at breast height in old-growth stands: A, Red spruce; B, balsam fir.

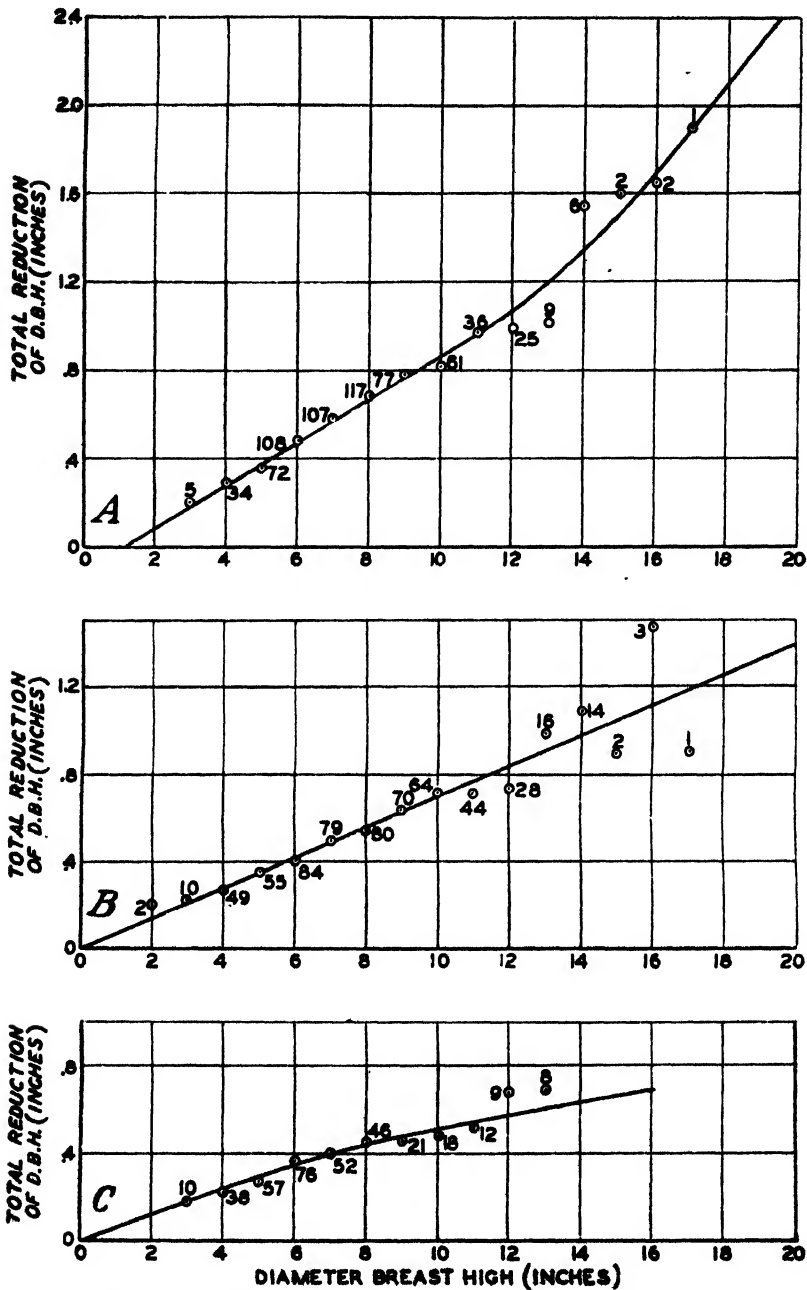


FIGURE 17.—Relation of total reduction of diameter breast high for bark thickness and butt swell to diameter breast high in even-aged stands: A, Red spruce; B, white spruce; C, balsam fir.

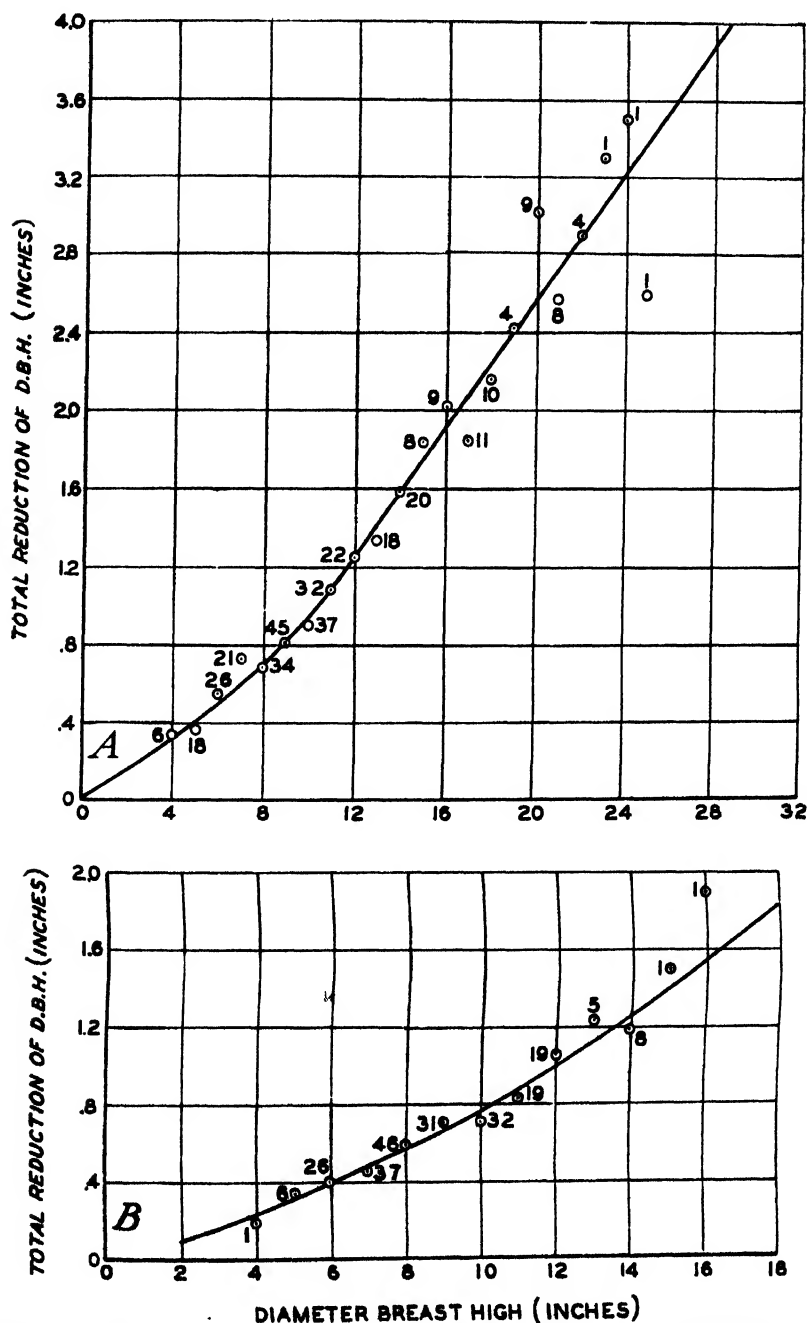


FIGURE 18.—Relation of total reduction of diameter breast high for bark thickness and butt swell to diameter breast high in old-growth stands: A, Red spruce; B, balsam fir.

TABLE 15.—Accuracy of estimates of total reduction of diameter breast high for bark thickness and butt swell

Type and species	Standard deviations of total reductions	Accuracy of estimate of total reduction			Standard error expressed as percentage of mean diameter breast high	Standard error expressed as percentage of volume of individual trees
		Standard error	Alienation index	Index of determination		
Even-aged stands:	Inches	Inches			Percent	Percent
Red spruce.....	0.34	0.20	0.61	0.63	2.6	5.3
White spruce.....	.27	.19	.71	.50	2.4	4.8
Balsam fir.....	.17	.14	.80	.36	2.0	4.0
Old-growth stands:						
Red spruce.....	.76	.39	.51	.74	3.5	7.0
Balsam fir.....	.32	.21	.66	.56	2.4	4.7

Since the influence of other factors was indicated in a general way in the discussion of butt swell and bark thickness, and since so much of the variability that can be accounted for is related to diameter breast high, it seemed desirable here to study the residuals from the curves of total reduction on diameter breast high rather than to use multiple correlation directly.

In the case of even-aged red spruce and white spruce there is a definite tendency for the difference between diameter breast high and normal diameter of trees of the same size class to be greater on poor sites than on good sites. But site index did not show any significant relationship in the old-growth stands or in even-aged balsam fir. Even in the case of even-aged red spruce, where the effect is most pronounced, the accuracy of estimate is increased less than 2 percent (a reduction of the alienation index from 0.61 to 0.59).

For even-aged balsam fir there is a definite relation with age that is not apparent in either of the other species. Balsam fir trees of the same size show a greater difference between diameter breast high and normal diameter in old stands than in younger ones. Trees of the same size would therefore have lower volumes in old stands than in younger ones, provided their form quotients were similar. But since form quotient tends to increase rapidly with age (p. 693) the effect of age on volume is compensating. The improvement of estimate of the total reduction of diameter breast high in even-aged balsam fir when age is taken into consideration amounts to about 16 percent, the alienation index being reduced from 0.80 to 0.64.

The relative significance of variations in bark thickness and butt swell as compared to variation in form quotient can be judged from the effect of each of these elements on estimates of tree and stand volume. Table 16 compares standard errors in tree volumes associated with the estimation of form quotient with those associated with estimates of butt swell and bark thickness. The former are 1.5 times the standard errors in estimation of form quotient from diameter, height, and crown percent (table 11), since a unit of form quotient is roughly equivalent to 1.5 percent of volume. The latter were derived by the approximate method of assuming the standard error expressed as percentage of diameter breast high to be equal to the standard error in inches (table 15) divided by the mean diameter of the trees in the sample, and then multiplying by 2, since it has been previously shown that volume errors will be double the percentage errors in diameter (2, p. 730).

TABLE 16.—Standard errors in estimation of volume of individual trees

Source of error	Even-aged stands			Old-growth stands	
	Red spruce	White spruce	Balsam fir	Red spruce	Balsam fir
Estimate of form quotient from diameter breast high, height, and crown percent.....	Percent 7.2	Percent 6.6	Percent 6.2	Percent 6.3	Percent 6.4
Estimate of butt swell and bark thickness from diameter breast high.....	5.3	4.8	4.0	7.0	4.7

In the estimation of individual trees variations in butt swell and bark thickness give rise to smaller volume errors than variation in form quotient except in the case of old-growth red spruce. But in interpreting these figures it must be borne in mind that volume tables are used to obtain aggregate volume of groups of trees or of stands and cannot be expected to give great accuracy for individual trees.

The standard error in volume of an entire stand associated with variations in butt swell and bark thickness would be reduced regularly in proportion to the square root of the number of trees tallied. But, if average form quotient is determined by any basis suggested in this paper or by actual measurement of a sample of trees, the standard error associated with variation in form quotient will never be reduced by more than the square root of the number of trees used in such determination, which is not likely to exceed 50.

It therefore appears that the spread between volume errors from these two sources would increase as the number of trees tallied increased. It seems quite certain that with these species, as was found to be the case with ponderosa pine (2, p. 737), the determination of average form quotient is a more important source of error than allowance for butt swell and bark thickness, but the latter approaches the former in significance in the larger sized old-growth timber.

CONCLUSIONS

When this study was commenced, it was believed that some adaptation of the form-class system of volume tables as developed in Sweden would prove superior to conventional tables based on diameter and height alone, since form-class tables take definite cognizance of differences in taper. The feasibility of obtaining the theoretical benefits of the form-class system in practice depends upon the soundness of the application of a single average form quotient to the entire stand, and upon ability to estimate the average form quotient satisfactorily and to determine the amount of bark thickness and butt swell. Furthermore, the superiority of tables derived by the form-class system will depend upon the extent to which the determinations of average form quotient and of the reduction of diameter breast high to normal diameter are superior to those that might be made from diameter and height, which are accounted for in tables of the conventional sort.

In this study of red spruce, white spruce, and balsam fir, variations in bark thickness and butt swell could not be consistently correlated with any factor other than diameter. This means that, so far as it will be practical to allow for this element, volume tables of the usual sort would be just as satisfactory as form-class tables.

In the analysis of variations in form quotient, also, diameter and height showed up as the most important factors. To the extent that variations in form quotient are associated with differences in diameter and height, conventional volume tables, if properly constructed and based on truly representative data, will automatically take care of the form-quotient problem. A measure of the superiority of form-class tables in respect to proper allowance for variations in form quotient may be obtained by a comparison of volume errors when form-quotient estimates are based on diameter, height, and crown percent (the most accurate and practical basis for evaluating form quotient developed in this study) with those which arise when form quotient is estimated from diameter and height alone, as is in effect the case with conventional tables. Measures of the accuracy of estimate of form quotient on these two bases are given in the last two lines of table 8, but they will have more meaning if expressed in percentage of volume, as in table 17.

TABLE 17.—Comparison of volume errors associated with different methods of evaluating form quotient

Type and species	Standard error in estimating form quotient from diameter breast high, and height ¹	Standard error in estimating form quotient from diameter breast high, height, and crown percent ¹	Improvement ¹
	Percent	Percent	Percent
Even-aged stands:			
Red spruce.....	8.3	7.2	1.1
White spruce.....	7.3	6.6	.7
Balsam fir.....	6.6	6.2	.4
Old-growth stands:			
Red spruce.....	7.0	6.3	.7
Balsam fir.....	7.4	6.4	1.0

¹ Expressed as percentage of volume.

It will be seen that even in this element no practical method of applying the form-class system yet devised offers prospect of giving more than 1 percent greater accuracy than might be expected from conventional tables for the species in question.

It may be argued further that, to the extent that form quotients within stands vary with size of the trees, the form-class system will be less accurate than conventional tables, since in the former a single average form quotient is usually applied to the entire stand whereas in the latter the form quotient varies automatically according to diameter and height.

On the whole, therefore, it may be concluded that in final application the form-class system must be on much the same footing as other volume-table methods, because differences in taper which can be evaluated in any practical way are so largely reflected in the diameter-height relationships. Methods of form-class analysis, however, still offer definite advantages in a more adequate and realistic technic, in reduction of number of trees required for reliable tables, in simplicity of compilation of tables in various units of product or standards of utilization, and in the possibilities for analytical comparison of different species or of timber of the same species under different growth conditions. In the latter field there is the greatest opportunity for future study and progress.

SUMMARY

Intensive study of the variation of form quotients and of bark thickness and butt swell has been undertaken to provide a clearer understanding of factors involved in the application of volume tables, with special reference to the use of the form-class system as developed by the writer in a previous paper. The study is based on measurements of 2,189 trees of red spruce, white spruce, and balsam fir covering a wide range of sizes, ages, densities, sites, and growth conditions in the Northeast. Punch-card machines were used in the manipulation of the data; and methods of multiple curvilinear correlation, as developed by Bruce and Reineke, were used in the analyses.

After graphic elimination of butt swell at breast height, the taper measurements for each locality were found to conform quite satis-

factorily to the formula for the stem curve: $\frac{x}{y} = \frac{x}{a+bx}$. Significant volume errors exceeded 2 percent in the case of a few samples only. White spruce gave the best fit. With red spruce the upper sections frequently fell below the formula, especially in old-growth stands. Even-aged balsam fir was similar to red spruce in fit, but in old-growth balsam fir diameters below the midpoint were consistently larger and those above the midpoint consistently smaller than the formula, with the effect on cubic volume tending to compensate.

The standard deviation of form quotients within the stands averaged not far from ± 4.5 units, which conforms to conclusions of previous work; but the variability was not closely associated with any of the factors which can be readily evaluated. However, the variation appeared to decrease with an increase of density, age, or form quotient.

The relation between form point and form quotient differed with each species and was not rectilinear. Although higher form quotients were generally associated with an increase in relative height of form point, very little if any increase in form quotient occurred after the form point went above 75 percent of the total height. In fact, tall trees with crowns of limited development often had relatively low form quotients.

Measurement of form point on about 45 trees is required to limit the standard error of the average form quotient to ± 1 unit. The use of form point has very little significance in old-growth red spruce and balsam fir.

A study of form quotients of the individual trees indicates that more than a third of the total variability of form quotient is associated with factors not included in this study and probably not readily susceptible to numerical evaluation. Of the numerical factors studied, diameter breast high, height, length of crown, and site index were the most important. Diameter and height appeared to have the greatest weight, but, these factors having opposite signs and being closely correlated, their effect was compensating. Form quotient increased rapidly with height until the trees were about 40 feet tall, after which there was little further increase. On the other hand, form quotients were at a maximum in trees about 7 inches in diameter and decreased steadily with increase in diameter. This negative correlation between diameter and form quotient, slight but significant,

appeared within individual stands as well as for the data as a whole. Similarly, within stands, dominant trees averaged lower in form quotient than codominants or intermediates and, as might be expected from this, trees with long crowns had lower form quotients than those with short crowns.

Length of crown is the only factor showing any appreciable crude correlation with form quotient when all the data for each species are thrown together. Form quotients generally reached a maximum in trees with crown lengths about 40 percent of total height. Estimates of form quotient can be made from length of crown with about the same accuracy as from form point.

Although trees on poor sites generally averaged higher in form quotient than those on better sites, the influence of site appeared to be absorbed in the diameter-height-crown length relationships. Alinement charts have been prepared for estimating form quotient of individual trees from diameter, height, and crown percent. Even using these three factors, about 40 trees are required to keep the standard error of the average form quotient down to ± 1 unit.

The average form quotient of even-aged stands increased rapidly up to an age of 70 years or a density of 1,200 trees per acre more than 3 inches in diameter, but beyond these points, little if any further increase took place. Alinement charts for estimating average form quotient of a stand from its age and density have also been prepared. Just as with individual trees, average form quotient can also be correlated with average crown length of dominant and codominant trees, and there is evidence that variations in average crown length are reflected and expressed approximately by the variations in age and number of trees per acre. For old-growth stands it appears that a single average form quotient may be safely applied upon inspection for certain easily recognized conditions of growth.

Where the species occurred in mixture, red spruce ran about four units of form quotient higher than either white spruce or balsam fir. Balsam fir and white spruce did not differ appreciably in form quotient when occurring together.

The amount of butt swell at breast height tended to increase with increase in diameter, but only a small proportion of the variation of butt swell can be accounted for by analysis of the numerical factors. A larger proportion of the variation in bark thickness can be accounted for, and this too is directly related to diameter. In trees of the species studied less than 12 inches in diameter, butt swell never amounted to more than half the double bark thickness. It exceeded bark thickness only in the case of old-growth red spruce larger than 17 inches diameter breast height.

Butt swell as determined by this study averaged 2 percent of the total tree volume in even-aged white spruce and $1\frac{1}{2}$ percent in even-aged balsam fir, regardless of size, while in even-aged red spruce the percentage of volume increased steadily, reaching 5 percent for trees 16 inches in diameter.

For practical purposes butt swell and bark thickness may be considered together. The total reduction of breast-high diameter can be estimated from diameter breast high alone, with a standard error not exceeding 0.2 inch in all cases except that of old-growth red spruce, yet the correlation is considerably closer in the case of old-growth red spruce than in any of the other sets of data. Although

there is some indication that the difference between diameter breast high and normal diameter is greater on poor sites than on good sites in even-aged red spruce and white spruce, little increase in accuracy of estimate can be obtained from consideration of this factor. In even-aged balsam fir the reduction of diameter breast high to normal diameter is definitely related to age, but the effect this would have on differences in volume for trees of the same size in stands of different ages tends to be offset by the fact that form quotients increase with age.

The determination of average form quotient is clearly shown to be a more important source of error in the estimation of volume of standing timber of the species studied than allowance for butt swell and bark thickness, but the latter approaches the former in significance in the larger sized old-growth timber.

Since so much of the variation in form quotient, bark thickness, and butt swell that can be accounted for is associated with diameter and height, form-class volume tables afford little advantage in actual use over properly constructed tables of conventional form. However, methods of form-class analysis do possess several technical advantages and offer good opportunity for analytical comparison of different species or of timber of the same species under different growth conditions.

LITERATURE CITED

- (1) BEAN, L. J.
1930. APPLICATION OF A SIMPLIFIED METHOD OF CORRELATION TO PROBLEMS IN ACREAGE AND YIELD VARIATIONS. *Jour. Amer. Statis. Assoc.* 25: 428-439, illus.
- (2) BEHRE, C. E.
1927. FORM-CLASS TAPER CURVES AND VOLUME TABLES AND THEIR APPLICATION. *Jour. Agr. Research* 35: 673-744, illus.
- (3) BRUCE, D., and REINEKE, L. H.
1931. CORRELATION ALINEMENT CHARTS IN FOREST RESEARCH: A METHOD OF SOLVING PROBLEMS IN CURVILINEAR MULTIPLE CORRELATION. *U. S. Dept. Agr. Tech. Bull.* 210, 88 pp., illus.
- (4) GEVORKIANTZ, S. R., and HOSLEY, N. W.
1929. FORM AND DEVELOPMENT OF WHITE PINE STANDS IN RELATION TO GROWING SPACE; A PRELIMINARY STUDY WITH FORM-CLASS VOLUME TABLES OF NATURAL AND PLANTED STANDS IN CENTRAL NEW ENGLAND. *Harvard Forest Bull.* 13, 83 pp., illus.
- (5) HEDEBY, R.
1929. EKENS STAMFORM OCH KURIKMASSA. [STEM-FORM AND VOLUME OF THE OAK.] *Svenska Skogsvårdsför. Tidskr.* 27: 123-232, illus. [In Swedish. Summary in English, pp. 229-232.]
- (6) HEIJBEL, I.
1929. SKOGSMATEMATISKA UNDERSÖKNINGAR RÖRANDE TALLENS BARK. [FOREST MATHEMATICAL RESEARCHES INTO THE PINE BARK.] *Svenska Skogsvårdsför. Tidskr.* 27: [269]-373, illus. [In Swedish. Summary in English, pp. 366-373.]
- (7) JONSON, T.
1912. TAXATORISKA UNDERSÖKNINGAR ÖFVER SKOGSTRÄDENS FORM. III. FORMBESTÄMNING Å STÅENDE TRÄD. *Svenska Skogsvårdsför. Tidskr.* 10: [235]-275, illus.
- (8) MEYER, W. H.
1929. YIELDS OF SECOND-GROWTH SPRUCE AND FIR IN THE NORTHEAST. *U. S. Dept. Agr. Tech. Bull.* 142, 52 pp., illus.
- (9) WRIGHT, W. G.
1927. TAPER AS A FACTOR IN THE MEASUREMENT OF STANDING TIMBER. *Canad. Forest Serv. Bull.* 79, 132 pp., illus.

THE RELATIONSHIP OF CERTAIN LEGUME MOSAICS TO BEAN¹

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INTRODUCTION

Since the virus of the common mosaic disease of bean (*Phaseolus vulgaris* L.) is seed-borne, the disease is widely distributed. Previous investigators (6, 11, 13)³ have shown that the amount of seed transmission of the virus varies; primary infected plants seldom produce more than 50 percent of infected seeds, and secondary infected plants produce an even smaller percentage. Notwithstanding the low percentage of seed-borne mosaic in most lots, the disease often becomes so wide-spread in the field, especially among the susceptible varieties, that all the plants may be infected by the end of the growing season.

Common bean mosaic is not equally severe in all sections of the country, which indicates, among other things, that climatic conditions may not be equally favorable for the manifestation of symptoms and that the agencies transferring the virus may be more numerous in some bean-growing sections than in others. There also may be hosts harboring other viruses that may be infectious to beans.

It has frequently been demonstrated and is generally understood that under field conditions the disease is spread by means of aphids from infected to healthy plants, but it has been doubted whether this is the sole source of virus transfer. Field observations have indicated that the source of some of the infection came from mosaic-infected legumes, both cultivated and wild. In some of the irrigated sections of the United States it was repeatedly observed that bean mosaic appeared more severe in the early part of the season along the borders of the field adjacent to fence rows, irrigation ditches, or roadways, which were often overrun with weeds, including several legumes. In a number of instances, 15 to 20 percent of infection was found in the outermost rows whereas only a trace was observed in the center of the field. This difference in severity of infection may have been due either to large aphid populations coming from the weeds transmitting certain of the related legume viruses to bean, or to the spreading of the common bean mosaic from one bean plant to another.

During the course of cross-inoculation studies with virus diseases of certain leguminous hosts it was noted that the virus extracts of some of these diseased hosts when inoculated to the bean produced symptoms unlike those of the common bean mosaic. It was shown that the viruses causing the mosaic diseases of pea (*Pisum sativum* L.), white clover (*Trifolium repens* L.), alsike clover (*T. hybridum* L.), white sweetclover (*Melilotus alba* Desr.), yellow sweetclover (*M. officinalis*

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³ Reference is made by number (italic) to Literature Cited, p. 748.

(L.) Lam.), alfalfa (*Medicago sativa* L.), and sweet pea (*Lathyrus odoratus* L.) are all transmissible to bean, whereas the virus producing the mosaic of red clover (*Trifolium pratense* L.) is not. Although a careful examination of the symptoms produced by the viruses of these legumes on bean reveals specific differences, the viruses from white clover, white sweetclover, yellow sweetclover, and alsike clover when inoculated to beans may produce under certain conditions symptoms that resemble the typical mottled and chlorotic symptoms of the common bean mosaic.

It is entirely probable that in the several legumes there may be present other mosaic viruses that may react differently from those reported here, notwithstanding symptomatological similarity. Furthermore, viruses from other mosaic-diseased legumes not included here may also produce on beans symptoms different from any so far reported.

In some cases certain viruses were inoculated only to the Stringless Green Refugee variety to determine their infectiousness. The viruses of pea, white clover, white sweetclover, alfalfa, and red clover mosaics were studied in some detail; those of alsike clover, yellow sweetclover, and sweet pea mosaics were not.

This paper describes the symptoms of these legume mosaic diseases on their normal hosts, together with the symptoms produced by these viruses when inoculated to bean, and compares differences in varietal reaction of the several legume mosaic diseases on bean varieties and other leguminous hosts with those of the common bean mosaic. Data on the comparison of the properties of these viruses are also given. An unknown virus obtained from a hybrid bean is compared with the common bean mosaic as to symptomatology and varietal susceptibility and resistance on a number of different bean varieties.

PREVIOUS INVESTIGATIONS

The early literature records few data regarding types or strains of the bean mosaic virus or the transmissibility of other legume mosaic viruses to bean. The early work of Reddick and Stewart (15, 16, 17, 18), Pierce and Hungerford (18), Fajardo (6, 7), and Nelson (11) dealt with the common bean mosaic merely as the disease affects the bean plant itself.

Numerous reports of mosaic diseases of other leguminous plants are recorded in the literature. Elliott (5) proved the existence of a mosaic disease of sweetclover and red clover and showed the virus to be infectious to these two hosts as well as to *Medicago arabica* (L.) Huds. and *Vicia faba* L. Dickson (3) reports successful reciprocal inoculations between the viruses of a number of clover mosaic diseases, but obtained negative results when cross inoculations to pea and bean were made. Carsner (2) proved that the virus causing curly top of sugar beets was infectious to beans. Doolittle and Jones (4) reported the transmission of a virus of garden pea to red clover and sweet pea. Böning (1) described a mosaic disease of broadbean (*Vicia faba*) whose virus was infectious to peas, crimson clover, and red clover. Wingard (21) showed that the tobacco ring spot virus was infectious to beans as well as to certain other legumes. Merkel (10) attempted to classify the bean mosaic into three types on the basis of symptomatology, and concluded that the mosaic dis-

eases of a number of Leguminosae which he examined were caused by the same virus. Fajardo (6) was unable to infect beans with the viruses from certain leguminous hosts and suggested that the mosaic disease of bean is perhaps specific to the bean plant. Price (14) showed that the tobacco mosaic virus was infectious to beans, producing local lesions on certain varieties. Weimer (19, 20) reported on a mosaic disease of alfalfa and its transmissibility, but did not record any cross-inoculation studies. Nelson (11) reported a virus disease of beans as being different from the common bean mosaic, and gave it the name of "rugose" mosaic. Zaumeyer (22) and Zaumeyer and Wade (24) recently reported on the transmissibility of a number of different legume mosaic diseases to bean and pea and showed that they were not the same as the common bean mosaic. Pierce (12) described another virus of bean which he stated is different from the common bean mosaic and which he designated as yellow bean mosaic.

MATERIALS

SOURCES OF VIRUSES

BEAN MOSAIC

The virus of the common bean mosaic used in the experiments was collected from diseased seedlings of the Stringless Green Refugee, a variety very susceptible to the disease.

PEA MOSAIC

One of the pea mosaic viruses was secured from infected Dwarf Telephone peas grown in southeastern Colorado in 1931. The virus extract from such plants when inoculated to bean produces typical and striking systemic mosaic symptoms, which are described later. This disease of pea, although difficult to distinguish on the basis of symptoms from the common pea mosaic, is distinct from it, and for the sake of clarity it will be referred to as pea mosaic virus 2, and that of the common pea mosaic as pea mosaic virus 1. Other pea mosaic material used for comparative purposes was collected in Maryland, Virginia, Montana, California, and Idaho. The lesions produced on beans from the material collected in Maryland were similar to those produced with the Colorado material, while the pea mosaic from the other States was similar to pea mosaic virus 1.

WHITE CLOVER MOSAIC

This virus was secured from material collected in the District of Columbia, Virginia, and Colorado. The reaction of the viruses from these three sources was identical; however, the experimental data presented in this paper are based on the virus from Virginia. The virus extract from this mosaic material produced both systemic and local lesions on certain bean varieties.

ALSIKE CLOVER MOSAIC

This mosaic material was collected near Rosslyn, Va., in the fall of 1933. Its difference from white clover mosaic was established by the reaction of Robust, Great Northern Idaho No. 1, and Corbett Refugee varieties.

WHITE SWEETCLOVER MOSAIC

Most of the specimens of this mosaic were collected in Maryland, but some were collected in Virginia, Colorado, and Wisconsin.⁴ The results obtained from all of these samples were identical.

YELLOW SWEETCLOVER MOSAIC

This virus was obtained from material collected near Rosslyn, Va., and Greeley, Colo. From the data obtained, it appears that this virus is identical with the virus of white sweetclover mosaic.

ALFALFA MOSAIC

The alfalfa mosaic described by Weimer (19, 20) was collected by him and sent to the writers in the fall of 1932. Other mosaic specimens were collected in Virginia and reacted identically with those of Weimer. As reported, this mosaic produces local necrotic lesions on beans. Pierce (12) later describes similar lesions produced by an alfalfa virus which he states appears to be distinct from the mosaic on the same species described by Weimer (19, 20).

RED CLOVER MOSAIC

The red clover mosaic virus was collected from diseased plants near Rosslyn, Va. It is believed that this virus is the same as the one described by Doolittle and Jones (4) as being infectious to garden peas and sweet peas.

SWEET PEA MOSAIC

This virus was extracted from infected plants collected in Colorado. It produced systemic infection when inoculated to beans and appears to be distinct from the mosaic described by Doolittle and Jones (4).

BEAN MOSAIC VIRUS 3

During the summer of 1932, in the examination of a large number of hybrid bean strains at Greeley, Colo., a very mild mosaic symptom was noted on a few plants of a hybrid in the F_7 generation between the Wells Red Kidney and Stringless Green Refugee varieties, a cross tolerant to the common bean mosaic. This virus produces distinctly different symptoms from those produced by the common bean mosaic on the susceptible bean varieties and is referred to here as bean mosaic virus 3.

SOURCES OF BEANS AND OTHER LEGUMES

The bean varieties used in the experimental work, the results of which are presented in the various tables, came from a number of sources. Many of them were furnished by reputable seed firms and others were grown by the writers. The Stringless Green Refugee used principally in these studies was grown by the writers, either under cloth or carefully rogued for mosaic. Corbett Refugee was furnished by the Sioux City Seed Co. This variety originated from a single plant selection of Stringless Green Refugee and is resistant

⁴ From Dr. R. A. Brink, University of Wisconsin, Madison, Wis.

to the common bean mosaic. Great Northern Idaho No. 1 was secured from the Idaho Agricultural Experiment Station. It is also resistant to the bean mosaic and is a selection from the commercial Great Northern bean. The Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, furnished seeds of other leguminous hosts.

METHODS

Previous investigators have reported difficulty in obtaining high percentages of infection with the common bean mosaic virus. Success in transmitting the disease depends on a number of conditions, such as the age of the plant to be inoculated, the age of the infected plant from which the virus is extracted, and the method of applying the inoculum to the plant. An abrasive such as quartz sand in the inoculum used by some other workers was not employed in these experiments because of the severe injury it causes to the inoculated plant. The inoculum was secured by grinding infected leaves in a mortar. When large quantities of the virus were desired, infected plants were passed through a meat grinder before extraction of the virus. The juice was then strained through a piece of cheesecloth and a little of the pulp was enclosed within a small square of cheesecloth and used as a pad for inoculation. In all cases the inoculations were made immediately after extraction, since most of the legume viruses lose their virulence rapidly outside the host.

The small pad saturated in the inoculum was applied to the upper surface of each primary leaf with brisk rubbing. Plants were inoculated when the primary leaves were fully expanded and the trifoliate leaves just unfolding. A block of wood about the size of the leaf to be inoculated was placed under it as a support, and before the block was used a small amount of the inoculum was rubbed on it. By rubbing the upper leaf surface with the pad saturated in the inoculum, the lower surface was slightly injured from the pressure against the supporting block. In this manner the inoculum was applied to both surfaces of the leaf, resulting in a relatively high percentage of infection.

SYMPTOMS

The mosaic disease commonly found on beans produces a large number of variable symptoms depending to a large extent on the variety infected. Even within a variety the symptoms may show decided variations. In such cases symptomatology alone would not be suitable for recognizing the identity of possible strains of the virus. Since the other legume mosaic viruses (pea virus 2, white clover, white sweetclover, alfalfa, and bean virus 3) when inoculated to bean produce symptoms that are distinctly different from the common bean mosaic as well as from one another, they can be separated on a basis of symptoms alone. There is a distinct varietal behavior of these viruses on beans, but the susceptible varieties always produce symptoms typical of the particular virus. Varieties more tolerant to the several viruses produce slightly different symptoms. The susceptibility or resistance to the mosaic viruses reported here of Corbett Refugee, Great Northern Idaho No. 1, and Robust resistant to the common bean mosaic appears to be an important feature in the differentiation of these viruses.

The descriptions of the symptoms produced by the viruses from the five different hosts are based on the symptoms as they occur on the Stringless Green Refugee beans. In a few instances they are described on Corbett Refugee and Robust.

COMMON BEAN MOSAIC

The symptoms of the common bean mosaic are variable, depending somewhat on the variety infected, the age of the plant, and the environmental conditions under which the plants are grown. Affected leaves show, in general, various degrees of mottling and chlorosis, downward cupping of the laminae, which gives an arched appearance to the leaflet, puckering, and blistering (fig. 1). Mosaic-infected leaves may be smaller than healthy ones and very much contorted. Frequently leaves show a darker green area along the midrib and lateral veins (fig. 1, *C* and *H*), together with varying degrees of mottling from only slight to almost complete yellowing (fig. 1, *A*). On the less susceptible varieties a ruffling or crinkling (fig. 1, *B*) of the leaves is characteristic of the disease, and this may be accompanied by a general chlorosis of the leaf with pronounced venation (fig. 1, *C*). Under greenhouse conditions the symptoms on these more tolerant varieties are usually not striking, but under field conditions severe infection may cause a stunting of the plant and reduction in leaf area.

PEA MOSAIC

It has been pointed out that there are at least two distinct forms of pea mosaic. In many respects the symptoms of the two forms are similar to each other and difficult to differentiate, but in other respects they may differ more widely. Both forms appear on the leaves and stipules and are first characterized by a faint mottling, the infected leaves being a lighter green than those of the normal plant. Later the mottling becomes more intense, owing to the presence of numerous dark-green areas, irregular in outline and occurring between the larger veins (fig. 2, *B*, *F*, and *G*).

In pea mosaic virus 2 a pronounced vein clearing appears later, and immediately adjacent to the larger veins the dark-green tissue often persists (fig. 2, *G*). The region between the veins remains green but is of a lighter shade than in the normal healthy plant. Especially along the periphery of the leaves, regions of yellow are often found. The leaves and stipules of the infected plant are smaller than normal, but there is only a slight waving and upward curling of the edges and wrinkling of the leaves. Infected plants are only slightly stunted, although the pods may be somewhat malformed and distorted and in some cases reduced in size.

Pea mosaic virus 1 differs from pea mosaic virus 2 in that there is only a slight vein clearing, with streaks of yellow found between the veins (fig. 2, *F*). The infected leaves and stipules are more wrinkled, puckered, and twisted, and smaller in size than those associated with pea mosaic virus 2. In later stages the uppermost leaves become rosetted with the tendrils malformed and knotted (fig. 2, *F*). In pea mosaic virus 2 infection this condition has not been observed. Infected plants are usually more stunted than those infected with pea mosaic virus 2.

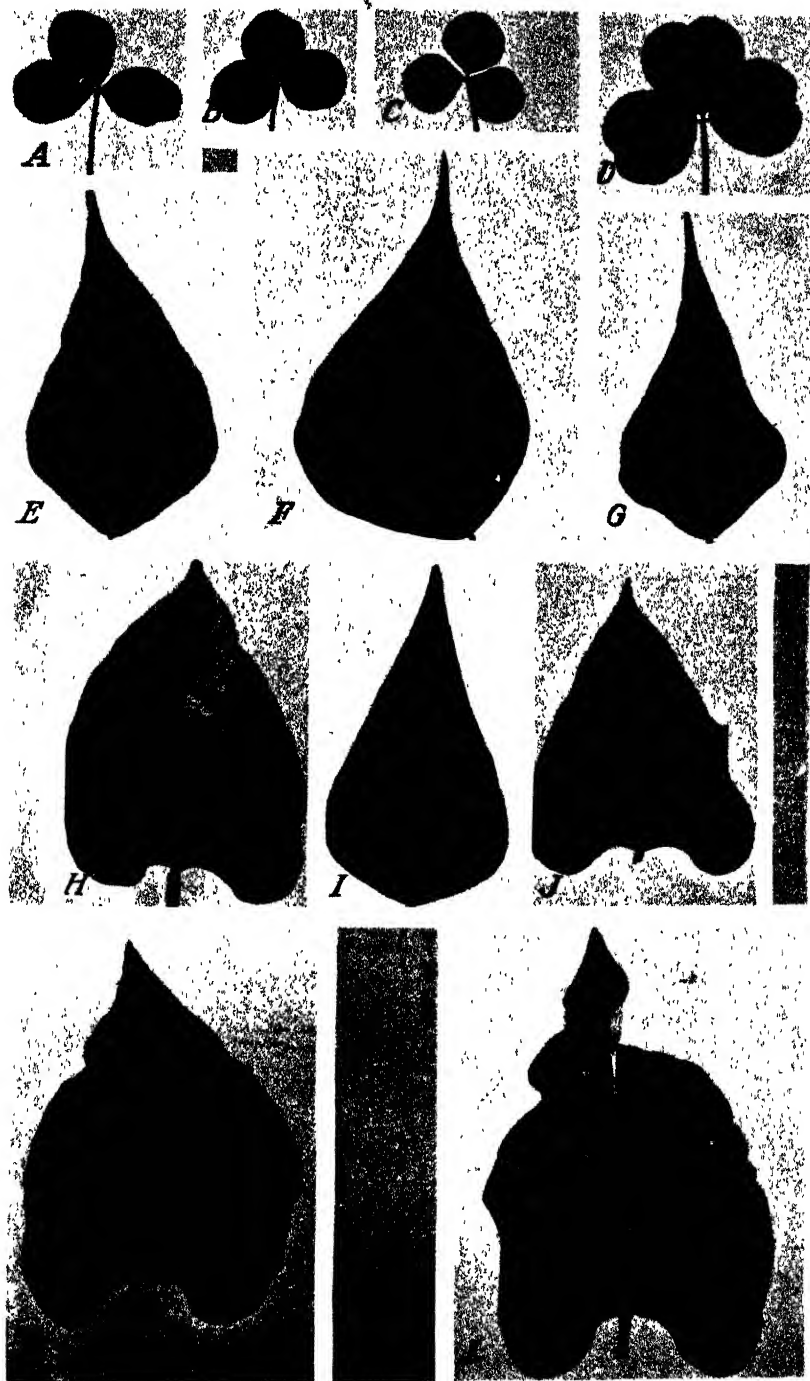


FIGURE 1.—Variations in leaf symptoms of the common bean mosaic on different varieties: *A* and *D*, White-seeded Refugee; *B*, Burpee Stringless Green Pod; *C*, Extra Early Refugee; *E*, Giant Stringless Green Pod; *F*, *H*, and *I*, Stringless Green Refugee; *G*, Refugee Wax.

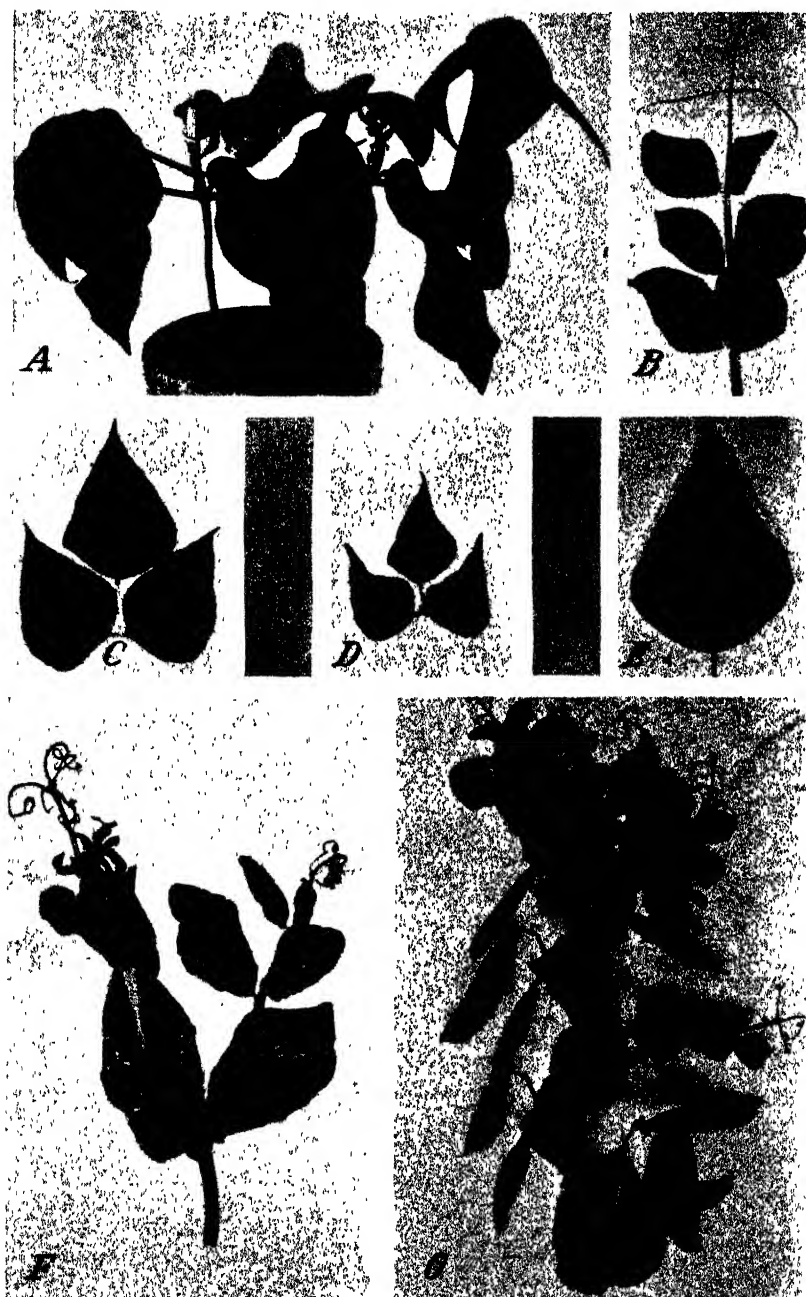


FIGURE 2.—Types of pea mosaic and the symptoms the virus produces when inoculated to beans: *A*, Stringless Green Refugee bean inoculated with pea virus 2; note drooping of leaflets and inward curling of youngest trifoliate leaves; *B*, pea virus 2 on Green Giant pea; *C* and *D*, pea virus 2 on Robust bean; *E*, healthy Robust bean; *F*, pea virus 1 on Dwarf Telephone pea; *G*, pea virus 2 on Dwarf Telephone pea.

PEA VIRUS 2 ON BEANS

The first symptom produced on beans by pea mosaic virus 2 is a drooping of the leaf and leaflets at the pulvini above the point of inoculation (fig. 2, *A*). This symptom is somewhat similar to that produced by the white sweetclover mosaic on beans. Later, numerous small yellow halolike chlorotic spots, varying in size from 1 to 5 mm, develop on these leaflets. The next formed trifoliate leaves appear to be thicker and smaller than the leaves of normal plants, and they droop and curve inward (fig. 2, *A*). Later, chlorotic spots similar to those produced on the first formed trifoliate leaves appear. The growing point of infected plants of susceptible varieties may be killed, and death of the entire plant may follow. Infected plants are usually decidedly stunted and very chlorotic.

Pea mosaic virus 1 was not infectious to beans.

WHITE CLOVER MOSAIC

White clover mosaic first manifests itself on the leaves of white clover as a slight mottle of light- and dark-green areas (fig. 3, *A*, *B*, *C*, and *D*). The darker green regions are usually found adjacent to the main vein and extending outward along the laterals, becoming lighter toward the margin of the leaflet. Occasionally the greater portion of the leaflet may be quite chlorotic with only the basal portion streaked with green. Islands of dark-green areas are frequently found in other portions of the leaf. Infected leaves are usually not distorted, but occasionally there is slight puckering and arching along the main vein. The symptoms are readily masked under certain environmental conditions.

WHITE CLOVER MOSAIC ON BEAN

When the white clover mosaic virus is inoculated to beans, it produces both local necrotic lesions and a systemic infection (fig. 3). Whether both symptoms are produced by the same virus or by two viruses has not been determined. The local lesions may appear from 36 to 48 hours after inoculation as small, somewhat circular, light-green areas, which at times may attain a diameter of from 2.5 to 5 mm (fig. 3, *H* and *L*). The smaller spots are circular with their edges only slightly irregular, whereas the large spots are seldom circular but are very irregular in outline. Later the lesions become brownish red, often accompanied by a slight clearing in the center which is usually surrounded by a dark ring of tissue, while outside of this is a region of lighter brown to red tissue. When the spots are very large with irregular borders there is no marked zonation of the lesions. If the lesions are numerous the uninfected normal green portions of the leaf turn yellow and die.

The systemic infection usually appears 10 to 11 days after inoculation and is manifested by blotches of light green or yellow areas on the trifoliate leaves, irregular in outline (fig. 3, *E*, *F*, and *G*). They are not bounded by the veins but extend throughout any portion of the lamina. These blotched areas may coalesce with similar areas, in some cases covering three-quarters of the leaf surface (fig. 3, *G*), leaving only small islands of normal green tissue. The infected leaves may show a slight ruffling, but their size is not reduced nor is

the leaf malformed as is often found with the common bean mosaic. Infected plants are not stunted, and under greenhouse conditions the

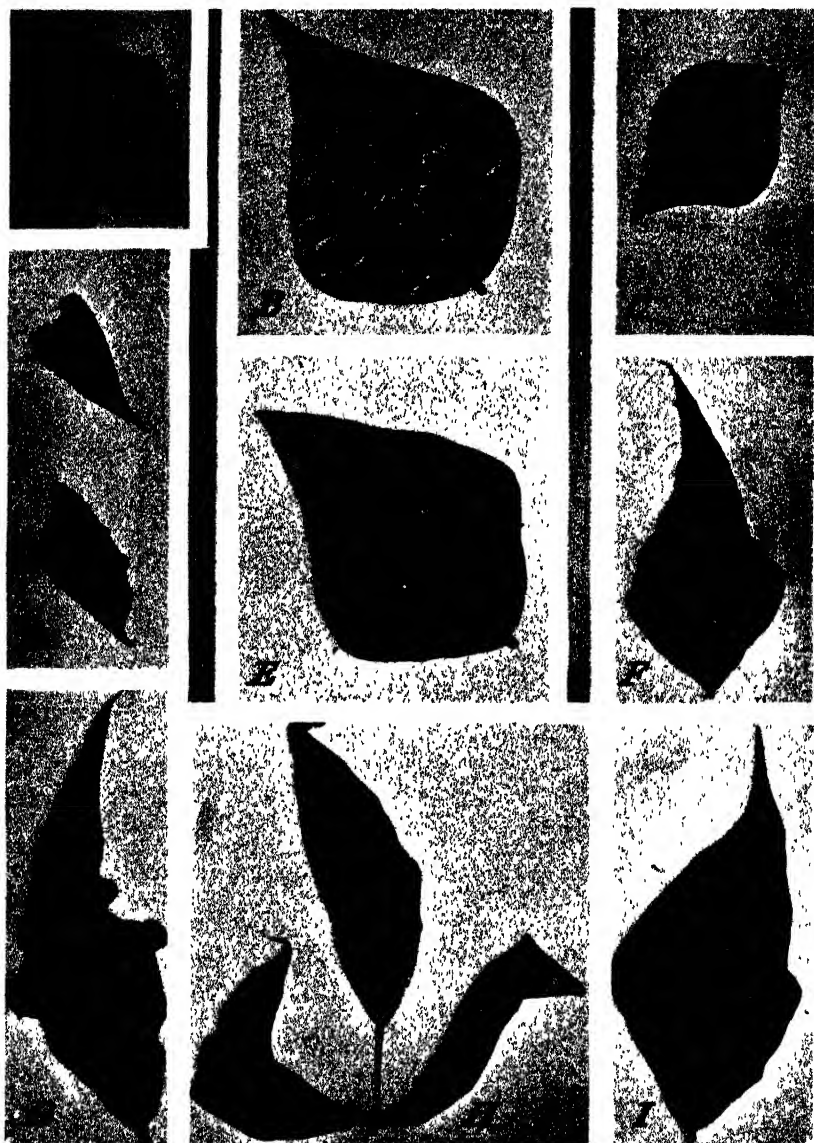


FIGURE 3.—White clover mosaic and the systemic and local symptoms the virus produces on different bean varieties: *A*, *B*, *C*, and *D*, White clover; *E*, trifoliate leaflet of Davis White Wax bean showing mottling; *F*, Pencil Pod Black Wax bean; *G*, Stringless Green Refugee bean; *H*, local lesions on Robust bean; *I* and *K*, healthy Stringless Green Refugee bean; *J*, healthy Robust bean; *L*, local lesions on Stringless Green Refugee bean.

symptoms may be readily masked. Under field conditions the symptoms might readily be mistaken for mild infections of the bean mosaic.

WHITE SWEETCLOVER MOSAIC

The symptoms of white sweetclover mosaic first appear as small light-yellow spots on the leaves (fig. 4, *F* and *G*). These spots may enlarge and coalesce with others, producing small light-green blotches interspersed with dark-green areas (fig. 4, *E*). Frequently there is a clearing of the veins, with the dark-green islands located between them. Severe infection may cause slight dwarfing and ruffling of the leaves. Under field conditions the infected plant may show a stunting in the early part of the year, but later in the summer and under high temperatures the symptoms become masked and the plant may be normal in size.

WHITE SWEETCLOVER MOSAIC ON BEAN

The white sweetclover mosaic virus produces very distinctive symptoms on beans. The first symptom appears about 1 week after inoculation and is a drooping of the leaf and leaflets at the pulvini. Later, small yellow, halolike chlorotic spots varying in size from 1.0 to 3.0 mm in diameter appear on these as well as on later formed leaves (fig. 4, *I*). The spots may be so numerous that they almost cover an entire leaflet and upon coalescing produce a pronounced chlorotic condition (fig. 4, *A* and *H*). Older leaves become roughened and chlorotic. Infected leaves may be stunted and distorted, especially if the infection is localized in one-half of the leaf (fig. 4, *B*). The diseased plants are smaller than the normal ones, but in no case has death resulted from infection.

ALFALFA MOSAIC

Alfalfa mosaic is first noticeable as small greenish or light-yellow streaks either along or between the veins (fig. 5, *B*, *C*, *D*, *E*, *F*, and *G*). These areas gradually become larger and the light areas more nearly yellow. In some cases only a portion of a leaflet may show symptoms. Such regions are usually bounded by a very irregular or jagged border of yellow, while the region within is lighter yellow in color. These areas may be either within the lamina or along the periphery of the leaflet. Oftentimes only 1 or 2 leaflets may show these symptoms, the third being normal in appearance. Another symptom that is found occasionally is manifested by concentric regions of yellow and green (fig. 5, *C* and *E*) which resemble the work done by the insect leaf miner.

ALFALFA MOSAIC ON BEANS

When inoculated to beans, the alfalfa mosaic virus produces small, reddish-brown, local lesions 2 days after inoculation (fig. 5, *A*). Some of the spots are surrounded by an irregular ring of tiny lesions of the same color. The larger type lesions vary from 0.5 to 2.0 mm in diameter. With age, the irregular ring of small lesions coalesces with the larger one and a clearing occurs in the center. The lesions differ from those produced by the white clover mosaic in that they are smaller in size and their edges are more regular. The disease does not become systemic.

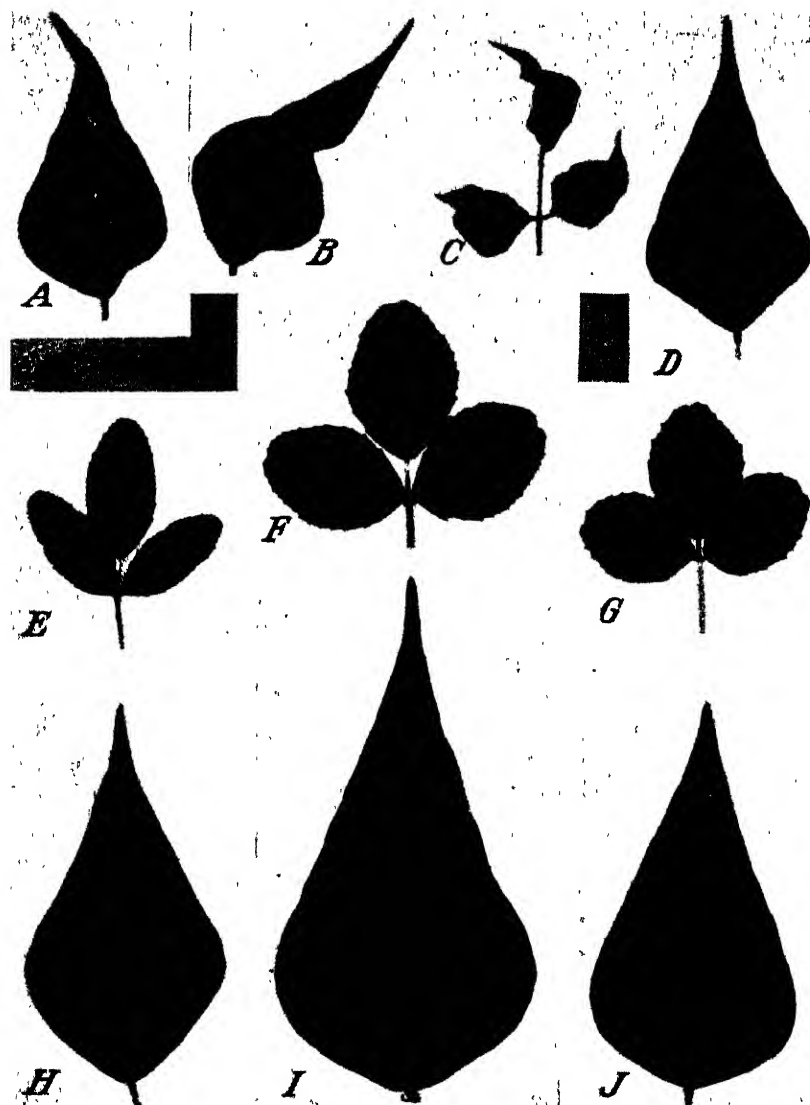


FIGURE 4.—White sweetclover mosaic and the symptoms produced by the virus on different bean varieties: *A*, Corbett Refugee bean showing mottling; *B*, Stringless Green Refugee bean showing distortion of leaflet; *C*, Blue Lake bean showing malformation; *D*, healthy Corbett Refugee bean; *E*, *F*, and *G*, different patterns of white sweetclover mosaic; *H* and *I*, Stringless Green Refugee bean; *J*, healthy Stringless Green Refugee bean.

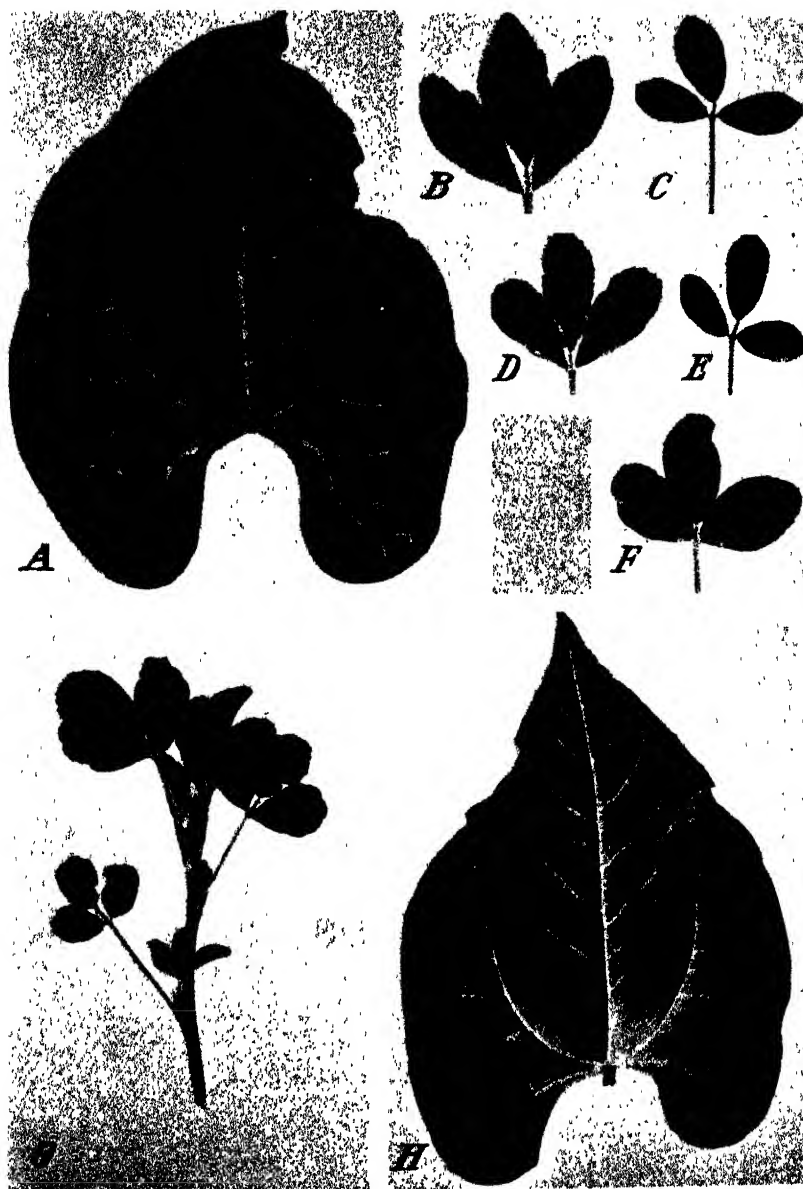


FIGURE 5.—Symptoms of alfalfa mosaic: *A*, Local lesions on bean (Stringless Green Refugee) produced by the virus of alfalfa mosaic; *B*, *C*, *D*, *E*, *F*, and *G*, different patterns of alfalfa mosaic; *H*, healthy Stringless Green Refugee bean.

RED CLOVER MOSAIC

Red clover mosaic appears as a faint mottling and yellow streaking of the leaves (fig. 6, *B*). The yellowing may cover a considerable portion of the leaf, leaving only islands of green which are frequently located adjacent to the veins. Infected plants are seldom stunted, and the symptoms are readily masked under conditions of high temperature.

Red clover mosaic was not infectious to beans.

RED CLOVER MOSAIC ON LEGUMES OTHER THAN BEANS

The leaflets of sweet peas infected with red clover mosaic show a pronounced mottling, pocking, and inward curling and occasionally some streaking. When the infection extends to the flowers there is a decided streaking, curling of the petals, and in many cases stunting.

Infected peas show a distinct mottling on the leaves and stipules very much like pea mosaic 1 and 2 (fig. 6, *F*.)

On the broadbean (*Vicia faba* L.) the infection is manifested by a very pronounced mottling and streaking of the leaves (fig. 6, *E*).

BEAN MOSAIC 3

Bean mosaic 3 appears as a very mild chlorosis on the hybrid of Stringless Green Refugee \times Wells Red Kidney. The symptoms might be overlooked, as no malformation of the leaves and no stunting of the plant are produced.

On Stringless Green Refugee bean the first symptoms produced by bean mosaic virus 3, 10 days after inoculation, are characterized by an extremely chlorotic and dwarfed condition of the trifoliate leaves immediately above the inoculated ones (fig. 7, *B*). Later formed leaves may show a very marked clearing of the veins and veinlets, bordered with normal green tissue. Later the remainder of the interveinal tissue turns yellow, resembling in general effect a calico pattern (fig. 7, *A*). The leaves of diseased plants are smaller than those of normal plants, but there is no wrinkling, curling, or other malformation. Infected plants of susceptible varieties may show a decided stunting.

RESISTANCE AND SUSCEPTIBILITY

Studies of previous workers (6, 13, 15, 16) on varietal resistance and susceptibility to the common bean mosaic virus, as well as the results obtained by the writers in these experiments, have shown that most bean varieties are susceptible but that they vary considerably in the degree of infection. Corbett Refugee, Robust, and Great Northern Idaho No. 1 were the only resistant varieties used that had been previously demonstrated to be immune to the common bean mosaic.

Transmission of the mosaic viruses of pea, white clover, white sweetclover, alfalfa, and bean mosaic virus 3 suggested the transmission to other varieties of beans to learn whether the behavior toward these mosaic diseases differed from that toward the common bean mosaic. Thirty varieties were inoculated under greenhouse conditions. Plants for this test were grown in greenhouse benches and inoculated, in the manner described previously, with an undiluted virus extract from the various mosaics, when the compound leaves

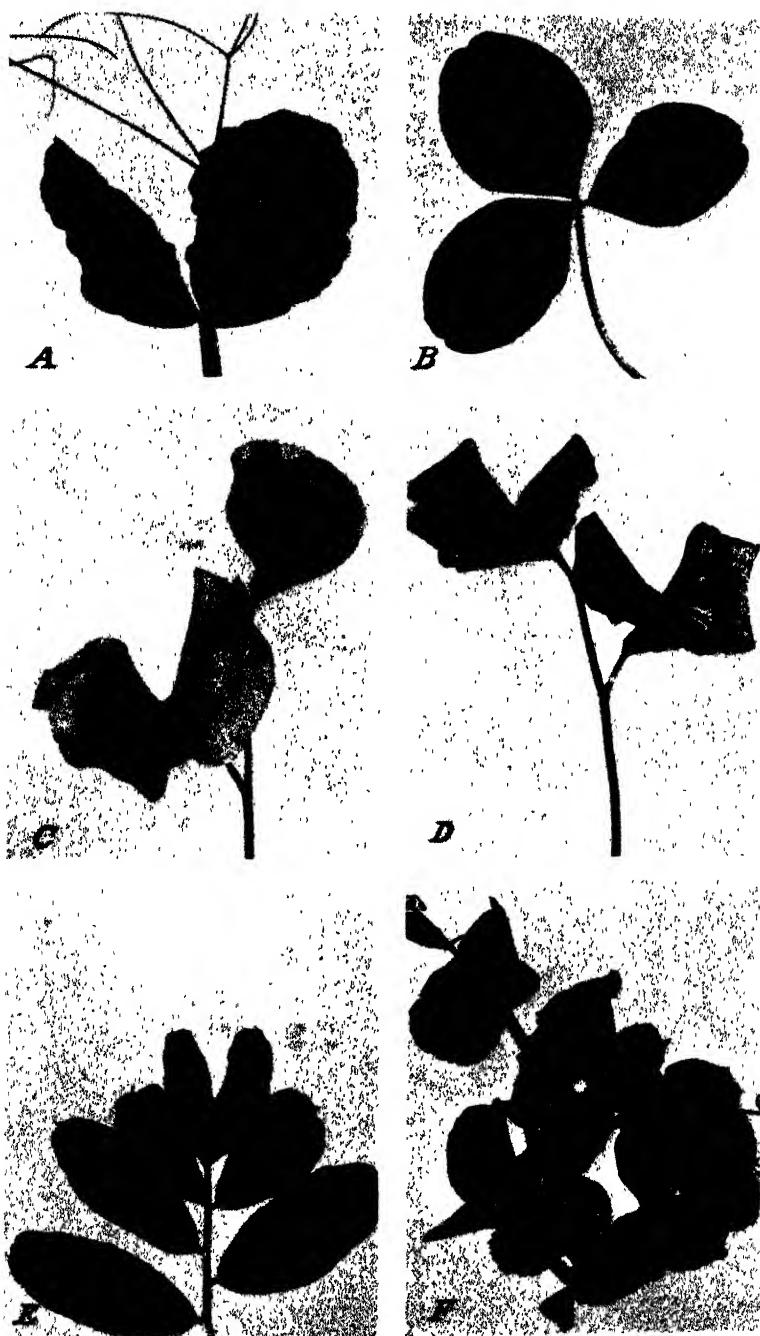


FIGURE 6.—Red clover mosaic and the symptoms produced by the virus on sweet pea, broadbean, and pea: A, Leaflets of sweet pea showing mottling; B, red clover mosaic; C, healthy sweet pea flowers (Baltimore Rose variety); D, infected sweet pea flowers showing streaking; E, broadbean showing mottling; F, pea (Dwarf Telephone variety) showing mottling.

were fully expanded. The viruses were extracted either from the mosaic-affected tissues of the original host plant or from beans infected with the several legume mosaic diseases. In either case the

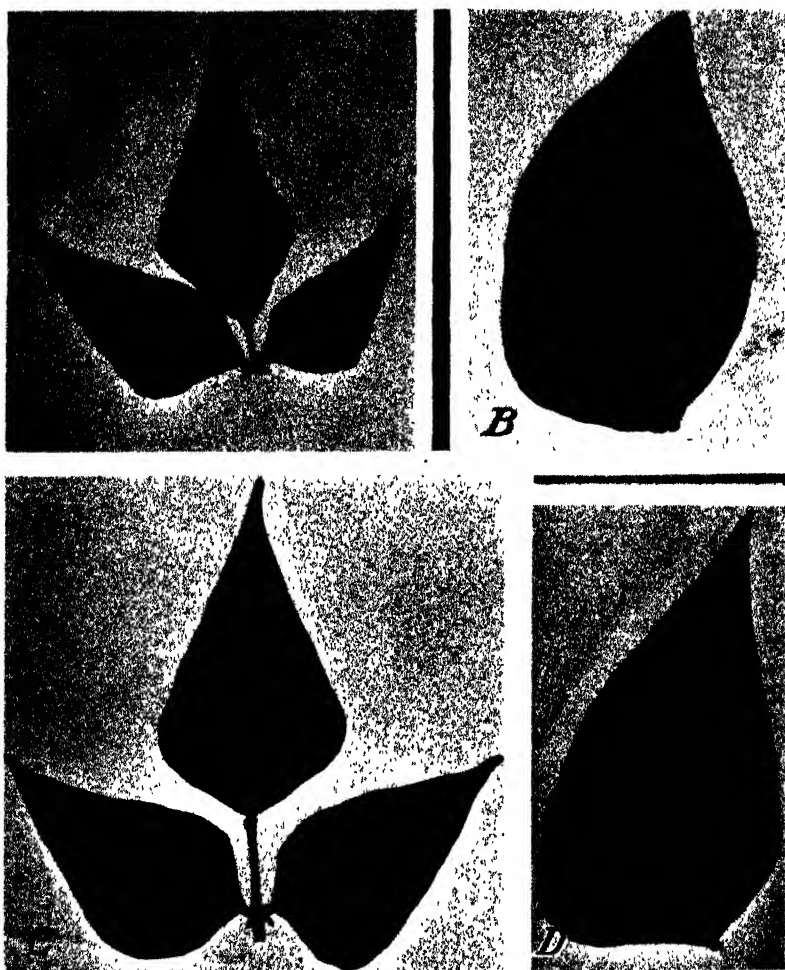


FIGURE 7.—Symptoms of bean mosaic virus 3 on Stringless Green Refugee bean: A. Trifoliate leaves showing extreme yellowing between the veins; B, leaf showing slight chlorosis; C and D, leaves from uninoculated plant.

results were comparable. The varieties listed in table 1 were not all grown and inoculated at the same time but were run in four series.

TABLE 1.—Comparative results of susceptibility and resistance of bean varieties to mosaic viruses of bean, pea, white clover, white sweetclover, alfalfa, and red clover, grouped according to susceptibility to the common bean mosaic

Group and variety	Results of inoculation with virus indicated ¹															
	Bean mosaic		Pea mosaic 2		White clover mosaic				White sweet-clover mosaic		Alfalfa mosaic, local lesions		Red clover mosaic			
					Systemic lesions		Local lesions									
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Group 1, very susceptible.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Dwarf Horticultural.....	10	10b	10	4a, 6b	10	5c	19	1	12	8c	10	10	10	7	0	0
Extra Early Red Valentine.....	10	8b	13	1a, 12b	18	8b, 2c	18	3	12	10c	9	7	10	7	0	0
Red Valentine.....	10	9b	10	1a, 4b	17	8b, 3c	18	7	10	8c	10	10	10	7	0	0
Refugee 1000-1.....	10	10b	10	10a	10	8b	10	8	12	9b	10	10	10	9	0	0
Refugee Wax.....	11	9b	10	5a, 5b	14	7b, 2c	14	0	10	8c	10	10	10	10	0	0
Stringless Green Refugee.....	15	15b	19	10a, 9b	24	23b	24	24	20	19b	10	10	15	15	0	0
Early Stringless Refugee.....	12	12b	10	10a	11	9b	11	7	10	8b	9	9	7	7	0	0
Group 2, moderately susceptible.																
Black Valentine.....	15	12c	17	6a, 11b	20	2b, 14c	20	0	11	5c	10	6	8	8	0	0
Blue Lake.....	10	10c	9	3a	19	4b, 1c	19	2	18	1c	10	10	10	7	0	0
Bountiful.....	9	9c	13	6a, 7b	16	5b, 11c	17	5	14	10c	10	10	10	9	0	0
Burpee Stringless Green Pod.....	12	11c	13	4a, 9b	16	8b, 4c	16	3	10	5c	9	8	10	10	0	0
Brittle Wax.....	13	12c	12	2a, 10b	16	12c	16	7	10	5c	10	10	10	10	0	0
California White.....	10	4c	9	3a	10	5b			10	0	10	10	8	0	0	0
Davis White Wax.....	10	10c	9	9b	15	7b, 7c	15	2	10	10c	10	7	9	0	0	0
French Horticultural.....	14	11c	12	8b, 4c	20	7b, 3c	20	5	14	12c	10	10	10	10	0	0
Full Measure.....	13	11c	10	2a, 8b	19	12b, 6c	19	11	10	8c	10	8	7	0	0	0
Giant Stringless Green Pod.....	12	11c	15	8b, 7c	17	14b, 1c	18	8	10	7c	10	10	9	0	0	0
Konserva.....	10	5c	16	2a, 6b	17	10c	17	4	14	7c	8	0	7	0	0	0
Longfellow.....	15	14c	11	2a, 9b	17	12b, 5c	17	3	14	10c	10	8	8	0	0	0
Low Champion Bush.....	14	11c	12	5a, 7b	17	11c	17	6	14	8c	10	10	10	10	0	0
New Stringless Green Pod.....	10	9c	13	2a, 11b	15	13b, 1c	15	4	10	5c	10	10	10	10	0	0
Pencil Pod Black Wax.....	14	13c	12	2a, 10b	19	12b, 4c	19	10	9	6c	10	10	10	8	0	0
Red Kidney (Dark Mahogany strain).....	11	7c	13	2a, 6b	18	0	21	9	20	0	8	8	6	0	0	0
Stringless Kidney Wax.....	10	9c	17	6a, 9b	20	3b, 3c	20	4	10	2c	9	9	8	0	0	0
Sure Crop Wax.....	10	9c	14	7b, 7c	13	8b	19	11	18	3c	10	10	7	0	0	0
Tendergreen.....	10	10c	10	7a, 3b	17	4b, 12c	15	7	10	9c	10	10	10	10	0	0
Tennessee Green Pod.....	10	10c	12	6a, 1b	17	4b, 1c	20	6	11	1c	7	2	10	0	0	0
Unrivalled Wax.....	13	2c	12	9c	18	6c	19	6	14	3c	14	6	9	0	0	0
Group 3, resistant:																
Corbett Refugee.....	15	0	15	3a, 12b	31	4b, 15c	31	0	35	4c	10	0	10	0	0	0
Great Northern Idaho No. 1.....	10	0	16	0	18	0	26	0	12	0	10	3	9	0	0	0
Robust.....	10	0	12	2a, 8b	22	7c	23	3	30	0	13	3	10	0	0	0

¹ a represents death of plant, b serious infection, c mild infection.

COMMON BEAN MOSAIC

In table 1 the varieties are grouped according to their relative susceptibility to the common bean mosaic. The 7 varieties in the first group are very susceptible; the 21 in the next group are moderately susceptible; and the 3 in the last group are resistant. It can be seen that in most cases a fairly high percentage of inoculated plants became infected in group 2 of the moderately susceptible varieties. But the symptoms produced were mild, whereas those in the first group (very susceptible) were severe. Under field conditions most varieties in the second group would not show a high percentage of infection and little if any stunting or reduced yield, while in the first group most of the varieties, especially the Refugee types, under ideal conditions and in some localities would show high percentages of disease with considerable stunting and reduced yield.

PEA MOSAIC VIRUS 2

All varieties of beans except Great Northern Idaho No. 1 were infected with pea mosaic virus 2 (table 1). In most of the inoculated varieties a percentage of the plants manifested severe symptoms and were killed. In the case of Refugee 1000-1 and Early Stringless Refugee all of the inoculated plants died. In group 1 there appears to be a correlation between the susceptibility of the different varieties to the pea mosaic virus 2 and to the common bean mosaic, because 100 percent infection was observed in practically all varieties except Red Valentine. In group 2 this correlation is not so evident. A very high percentage of infection occurred with the production of severe symptoms except in the case of Unrivalled Wax. This variety was the only one in this group that showed mild symptoms. Great Northern Idaho No. 1, which is resistant to the viruses of both the common bean mosaic and pea mosaic 2, offered the only point of similarity in group 3. The Corbett Refugee and Robust varieties showed extreme susceptibility to pea mosaic virus 2.

WHITE CLOVER MOSAIC VIRUS

Thirty-one varieties of beans were inoculated with the white clover mosaic virus, and only the Great Northern Idaho No. 1 was resistant. The Red Kidney (Dark Mahogany strain) alone showed only local lesions, with no systemic infection. Twenty-two varieties manifested very marked symptoms, while 6 varieties were only slightly infected. In addition to the mottled mosaic symptoms produced on the susceptible varieties, local lesions were produced on 25 varieties. The varieties resistant to the local lesions were Refugee Wax, Black Valentine, Corbett Refugee, and Great Northern Idaho No. 1.

In group 1 all the varieties showed very marked symptoms of the systemic infection except Dwarf Horticultural, which was only mildly infected. In group 2 Red Kidney (Dark Mahogany strain) was resistant to the systemic infection but susceptible to the local lesions. Although the percentage of infection was quite high in most cases, the symptoms produced were severe in 16 varieties and mild in 4. In group 3 Great Northern Idaho No. 1 was resistant, while Corbett Refugee and Robust were susceptible, the latter variety being less susceptible than the former. Regarding the production of local lesions, all varieties in group 1 were susceptible except Refugee Wax. Dwarf Horticultural and Extra Early Red Valentine were only mildly infected. In group 2 only Black Valentine was resistant, while the other varieties were only mildly susceptible. In group 3 Corbett Refugee and Great Northern Idaho No. 1 were resistant, while Robust was susceptible to the local lesions. There appears to be no complete correlation between the systemic and the local lesions produced by the white clover mosaic virus on beans.

WHITE SWEETCLOVER MOSAIC VIRUS

The white sweetclover mosaic virus infected 27 out of 31 varieties. The 4 resistant varieties were California White, Red Kidney (Dark Mahogany strain), Great Northern Idaho No. 1, and Robust. Three varieties were severely infected, while 24 showed milder symptoms. All the severely infected varieties were of the Refugee type found in

group 1. Refugee Wax, however, was mildly infected. In group 2 most of the varieties were mildly infected. Although many of the varieties in group 1 showed mild symptoms, a high percentage of the inoculated plants became infected. In group 2 most of the varieties showed lower percentages of infection in addition to the weak symptoms produced. In group 3 the only susceptible variety was the Corbett Refugee.

ALFALFA MOSAIC VIRUS

Alfalfa mosaic virus was infectious to all varieties except Corbett Refugee and Konserva. It produced only local lesions.

BEAN MOSAIC 3

The data in table 2 show that in general the mosaic virus from the hybrid bean produces more severe symptoms on beans than does the common bean mosaic. There appears to be no correlation between this virus (bean mosaic 3) and the one causing the common mosaic of beans. Two varieties (Great Northern Idaho No. 1 and Robust) that are resistant to bean mosaic were also resistant to bean mosaic 3,

TABLE 2.—Comparative susceptibility and resistance of bean varieties to the viruses of the common bean mosaic and bean mosaic 3, grouped according to the susceptibility to the common bean mosaic

Group and variety	Results of inoculation with virus indicated ¹			
	Common bean mosaic		Bean mosaic 3	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	Number	Number	Number
Group 1, very susceptible:				
Dwarf Horticultural.....	10	10b	12	8c
Red Valentine (Extra Early).....	10	8b	10	5c
Red Valentine.....	10	9b	10	0
Refugee 1000-1.....	10	10b	10	7b
Refugee Wax.....	11	9b	9	8b
Stringless Green Refugee.....	15	15b	10	8b
Stringless Refugee (Early).....	12	12b	8	6b
White Seeded Refugee.....	10	10b	10	6b
Group 2, moderately susceptible:				
Black Valentine.....	15	12c	9	4c
Blue Lake.....	9	3c	10	0
Bountiful.....	9	9c	10	10b
Burpee Stringless Green Pod.....	12	11c	10	10b
Brittle Wax.....	13	12c	10	4c
California White.....	10	4c	10	0
Davis White Wax.....	10	10c	10	10b
French Horticultural.....	14	11c	10	2b
Full Measure.....	13	11c	9	5c
Giant Stringless Green Pod.....	12	11c	10	10b
Konserva.....	10	5c	9	0
Longfellow.....	15	14c	9	7c
Low Champion Bush.....	14	11c	11	8b
New Stringless Green Pod.....	10	9c	10	10c
Pencil Pod Wax.....	14	13c	10	4c
Red Kidney (Dark Mahogany strain).....	11	7c	8	5c
Stringless Kidney Wax.....	10	9c	11	2b
Sure Crop Wax.....	10	9c	14	8b
Unrivalled Wax.....	13	2c	9	5c
Group 3, resistant:				
Corbett Refugee.....	15	0	16	5b
Great Northern Idaho No. 1.....	10	0	15	0
Robust.....	10	0	13	0

¹ b represents serious infection; c, mild infection.

while Corbett Refugee, which is resistant to the former, was susceptible to the latter. Red Valentine, Blue Lake, California White, and Konserva, all susceptible to the common bean mosaic, were resistant to bean mosaic 3. This virus produced severe symptoms on 14 of the 30 varieties tested and mild infection on 10, while 6 were resistant. The common bean mosaic produced severe symptoms on 8 varieties and mild infection on 20, while 3 varieties were resistant.

COMPARISON OF LOCAL LESIONS PRODUCED BY WHITE CLOVER AND ALFALFA VIRUSES WITH THOSE PRODUCED BY OTHER VIRUSES ON BEANS

The production of local necrotic lesions on beans by a virus has been described previously by Wingard (21), who reported the tobacco ring spot virus producing local lesions, although no description of the symptoms was given. Price (14) records the development of local lesions due to tobacco mosaic on 16 varieties of beans. Zau-meyer and Wade (24) reported the production of local lesions on beans inoculated with the mosaic virus of white clover and alfalfa. Pierce (12) compared the resistance and susceptibility of beans to the viruses of tobacco mosaic, tobacco ring spot, and alfalfa mosaic.

In order to obtain a comparison between the local lesions produced by tobacco mosaic and tobacco ring spot with those produced by the viruses from alfalfa and white clover, the work of Price (14) and Pierce (12) was repeated in part. Twenty bean varieties were inoculated with the viruses of tobacco mosaic and tobacco ring spot. The tobacco mosaic produced symptoms identical with those described by Price (14). The symptoms due to the tobacco ring spot virus agreed with those described by Pierce (12). They appear on the inoculated leaves as necrotic lesions which gradually coalesce with similar spots and finally cause death of the inoculated leaf of certain varieties. The infection later becomes systemic with the final death of the plant. All varieties tested reacted similarly.

The local lesions produced by the alfalfa mosaic virus were brownish red in color, while those produced by the tobacco mosaic virus were dark brown. The lesions caused by the tobacco mosaic virus, according to Price (14) and confirmed by the writers, are never more than one-half to 1 mm in diameter, whereas those of alfalfa reached a diameter of 2 mm.

The local lesions produced by the white clover mosaic virus on beans are not identical with those produced by the other two viruses. As mentioned previously, they are larger ($2\frac{1}{2}$ to 5 mm in diameter) than those produced by the tobacco and alfalfa mosaic viruses. They may or may not be circular, and the borders are irregular in outline.

Table 3 records the data on the varietal susceptibility and resistance of beans to the viruses of the four hosts mentioned above, which produce local lesions on beans. A complete list of bean varieties is not given, but only those that were inoculated with the four viruses. A more complete list of the varietal reactions to the local lesions of the viruses of white clover mosaic and alfalfa mosaic is given in table 1. The resistance and susceptibility of the varieties to the viruses of tobacco mosaic and tobacco ring spot agree with the data presented by Price (14) and Pierce (12).

TABLE 3.—Comparative varietal behavior of bean varieties with regard to local lesions produced by the viruses of white clover mosaic, alfalfa mosaic, tobacco mosaic, and tobacco ring spot

Variety	Reaction to virus of—			
	White clover mosaic	Alfalfa mosaic	Tobacco mosaic	Tobacco ring spot
Black Valentine.....	Resistant.....	Susceptible.....	Resistant.....	Susceptible.....
Bountiful.....	Susceptible.....	do.....	do.....	Do.....
Burpee Stringless Green Pod.....	do.....	do.....	do.....	Do.....
Corbett Refugee.....	Resistant.....	Resistant.....	Susceptible.....	Do.....
French Horticultural.....	Susceptible.....	Susceptible.....	Resistant.....	Do.....
Full Measure.....	do.....	do.....	Susceptible.....	Do.....
Giant Stringless Green Pod.....	do.....	do.....	Resistant.....	Do.....
Great Northern Idaho No. 1.....	Resistant.....	do.....	Susceptible.....	Do.....
Konserva.....	Susceptible.....	Resistant.....	Resistant.....	Do.....
Low Champion Bush.....	do.....	Susceptible.....	do.....	Do.....
New Stringless Green Pod.....	do.....	do.....	do.....	Do.....
Pencil Pod Black Wax.....	do.....	do.....	do.....	Do.....
Robust.....	Resistant.....	do.....	Susceptible.....	Do.....
Red Kidney (Dark Mahogany strain).....	Susceptible.....	do.....	Resistant.....	Do.....
Refugee Wax.....	Resistant.....	do.....	Susceptible.....	Do.....
Stringless Green Refugee.....	Susceptible.....	do.....	do.....	Do.....
Stringless Kidney Wax.....	do.....	do.....	Resistant.....	Do.....
Sure Crop Wax.....	do.....	do.....	do.....	Do.....
Tennessee Green Pod.....	do.....	do.....	do.....	Do.....
Unrivalled Wax.....	do.....	do.....	Susceptible.....	Do.....

Decided variations are noted in the susceptibility of the different varieties of beans to the various mosaic diseases producing local lesions (table 3). Of the 20 varieties inoculated, 7 are susceptible to the tobacco mosaic virus, all to the ring spot virus, 18 to the alfalfa virus, and 15 to the white clover virus.

Three varieties—Full Measure, Stringless Green Refugee, and Unrivalled Wax—were susceptible to the four viruses. Corbett Refugee, which was resistant to the alfalfa mosaic virus, was susceptible to the viruses of tobacco mosaic and tobacco ring spot. Konserva, also resistant to the alfalfa virus, was resistant to the tobacco virus but susceptible to the tobacco ring spot virus. No variety studied was resistant to all four of the viruses.

TRANSMISSION TO OTHER LEGUMINOSAE

In addition to inoculating a large number of bean varieties, several other species of the genus *Phaseolus*, as well as species in other genera, were inoculated with the viruses of the common bean mosaic, pea mosaic 2, white clover mosaic, white sweetclover mosaic, alfalfa mosaic, and red clover mosaic. The following Leguminosae were inoculated with these viruses from the regular hosts: Pigeonpea (*Cajanus indicus* Spreng.), knife bean or swordbean (*Canavali gladiata* (Jacq.) DC.), chickpea (*Cicer arietinum* L.), hyacinth-bean (*Dolichos lablab* L.), sweet pea (*Lathyrus odoratus* L.), lentil (*Lens esculenta* Moench), white lupine (*Lupinus albus* L.), alfalfa (*Medicago sativa* L.), white sweetclover (*Melilotus alba* Desr.), tepary bean (*Phaseolus acutifolius latifolius* Freeman), adzuki bean (*P. angularis* (Willd.) W. F. Wight), mung bean (*P. aureus* Roxb.), rice bean (*P. calcaratus* Roxb.), lima bean (*P. lunatus* Benth.), civet bean (Henderson bush variety) (*P. lunatus* L.), urd bean (*P. mungo* L.), pea (*Pisum sativum* L.), soybean (*Soja max* (L.) Piper), velvetbean (*Stizolobium deeringianum* Bort), white clover (*Trifolium repens* L.)

red clover (*T. pratense* L.), broadbean (*Vicia faba* L.), vetch (*V. americana* Muhl.), asparagus-bean (*Vigna sesquipedalis* (L.) Frurwirth), and cowpea (*V. sinensis* (L.) Endl.). The plants were inoculated in the usual manner by rubbing the leaves with the different virus extracts. Table 4 gives the results of these inoculations.

TABLE 4.—Susceptibility of various legumes to the mosaic viruses of the common bean, pea 2, white clover, white sweetclover, alfalfa, and red clover

Host	Reaction to virus of— ¹											
	Pea mosaic 2		White clover mosaic		White sweetclover mosaic		Alfalfa mosaic		Red clover mosaic		Common bean mosaic	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
<i>Cajanus indicus</i> (pigeon-pea).....	9	0	9	0	8	2	8	0	10	0	9	0
<i>Canavalia gladiata</i> (knife bean).....	5	0	6	0	4	0	5	0	6	0	6	0
<i>Cicer arietinum</i> (chick-pea).....	11	5	19	7	19	15	10	0	22	5	10	0
<i>Dolichos lablab</i> (hyacinth-bean).....	10	0	20	0	10	0	9	0	10	0	10	0
<i>Lathyrus odoratus</i> (sweet pea, Baltimore Rose variety).....	10	6	20	20	18	17	10	10	20	19	14	0
<i>Lens esculenta</i> (lentil).....	15	10	23	22	24	19	-----	-----	18	18	23	0
<i>Lupinus albus</i> (white lupine).....	10	0	9	0	10	0	9	0	8	0	8	0
<i>Medicago sativa</i> (alfalfa).....	5	0	5	2	5	0	5	3	5	0	5	0
<i>Medicago alba</i> (white sweetclover).....	9	5	8	7	9	9	10	8	12	0	10	0
<i>Phaseolus acutifolius latifolius</i> (tepary bean).....	10	0	8	0	8	0	10	0	8	0	8	0
<i>P. angularis</i> (adzuki bean).....	8	7	9	0	10	0	10	8	11	0	10	0
<i>P. calcaratus</i> (rice bean).....	18	0	19	0	17	0	16	6	18	0	9	0
<i>P. aureus</i> (mung bean).....	10	6d	15	12d	12	4d	10	6d	14	14d	10	0
<i>P. lunatus macrocarpus</i> (lima bean).....	9	0	10	0	9	0	10	0	8	0	10	0
<i>P. lunatus</i> (civet bean, Henderson bush variety).....	8	0	10	8	9	0	8	0	8	0	6	0
<i>P. mungo</i> (urd bean).....	10	0	8	5d	10	0	9	2d	10	0	8	0
<i>Pisum sativum</i> (pea, Dwarf Telephone variety).....	15	8	20	20	20	20	10	0	15	9	20	0
<i>Soja max</i> (soybean).....	10	0	11	0	12	0	8	0	12	0	14	0
<i>Stizolobium deeringianum</i> (velvet bean).....	7	0	8	0	6	0	6	0	8	0	7	0
<i>Trifolium repens</i> (white clover).....	5	3	5	3	5	0	5	0	5	0	5	0
<i>T. pratense</i> (red clover).....	5	0	5	5	5	5	5	3	5	2	5	0
<i>Vicia faba</i> (broadbean).....	10	8	10	10	10	10	9	6	10	8	10	0
<i>V. americana</i> (vetch).....	12	0	10	10	10	9	10	6	12	11	8	0
<i>Vigna sesquipedalis</i> (asparagus-bean).....	10	0	11	0	12	0	9	0	10	0	10	0
<i>V. sinensis</i> (cowpea).....	10	0	9	0	10	0	10	0	9	0	10	0

¹ d represents local lesions.

Previous investigators have tested many of these species with the common bean mosaic. Reddick and Stewart (15) report *Phaseolus lunatus macrocarpus*, *P. acutifolius latifolius*, and *Vicia faba* as being susceptible to the common bean mosaic. Fajardo (?) was unable to transmit the virus of bean mosaic to other leguminous hosts. Nelson (11) found that in addition to the three species reported by Reddick

and Stewart the following were also susceptible to the common bean mosaic: *Phaseolus calcaratus*, *P. lunatus*, *P. angularis*, *P. coccineus*, and *Vigna sesquipedalis*. He did not inoculate these hosts artificially, but relied on natural insect dissemination in the field.

Pierce (12) confirmed the results of Reddick and Stewart (15) and also some of those reported by Nelson and in addition was able to infect *Phaseolus aureus*, *Lespedeza striata* (Thunb.) Hook. and Arn., and *Vicia sativa* L.

The writers have been unable to confirm the results of these investigators, which may have been due to differences in varieties within a species. *L. striata* and *V. sativa* were not used in these tests. In a number of instances mild mottling was observed but the symptoms were not definite enough to establish their susceptibility.

As can be seen from table 4, the host range of the several virus diseases shows considerable variation. Pea mosaic virus 2 infects *Phaseolus angularis*, *P. aureus*, *Cicer arietinum*, *Lathyrus odoratus*, *Lens esculenta*, *Melilotus alba*, *Pisum sativum*, *Trifolium repens*, and *Vicia faba*.

White clover mosaic was infectious to *Phaseolus aureus*, *P. lunatus*, *P. mungo*, *Cicer arietinum*, *Lathyrus odoratus*, *Lens esculenta*, *Medicago sativa*, *Melilotus alba*, *Pisum sativum*, *Trifolium repens*, *T. pratense*, *Vicia faba*, and *V. americana*.

The hosts susceptible to white sweetclover mosaic are the same as those of white clover mosaic except that the virus of white sweetclover also infected *Cajanus indicus* but not *Trifolium repens*, *Medicago sativa*, or *Phaseolus lunatus*.

Alfalfa mosaic virus showed variations in host susceptibility in comparison with the viruses of white clover and white sweetclover. Infection was secured on *Phaseolus angularis*, *P. calcaratus*, *P. aureus*, *P. mungo*, *Lathyrus odoratus*, *Medicago sativa*, *Melilotus alba*, *Trifolium pratense*, *Vicia faba*, and *V. americana*.

The red clover mosaic virus reacted similarly to the viruses of white clover and white sweetclover. It was infectious to all of the hosts that were susceptible to the viruses of these two clovers, with the exception of *Phaseolus lunatus*, *P. mungo*, *Cajanus indicus*, *Medicago sativa*, *Melilotus alba*, and *Trifolium repens*.

The various hosts reacted differently to the several viruses. The viruses of pea mosaic 2, white clover, white sweetclover, and red clover were, in general, infectious to the same hosts. The alfalfa mosaic virus varied from these four viruses in host behavior.

The viruses of pea mosaic 2, white clover, white sweetclover, and red clover caused death to both *Cicer arietinum* and *Lens esculenta* without the production of any distinctive mottle symptom. On *Phaseolus aureus* and *P. mungo* the mosaic viruses of pea 2 and of these three clovers produced local lesions on the inoculated leaves. The symptoms first appeared as small irregular brown spots from 0.5 to 2 mm in diameter. Later the lesions coalesced, forming large irregular blotches slightly darker in color than in the earlier stages, and covering in some cases as much as one-fourth of the leaf area.

In general, the symptoms produced on a single host by the various viruses are alike. However, the several viruses when inoculated to the various hosts may produce decidedly different symptoms; as, for example, pea mosaic 2 produces local lesions on *Phaseolus aureus*

and systemic lesions on the other hosts that it infects. It appears that none of the species used in these inoculations can be used successfully as differential hosts in readily distinguishing the several viruses, a method that has been shown to be very effective and helpful in the separation of the mosaic virus complexes of other host plants.

Most of the outstanding differences in host reaction of the several viruses are noted in table 5.

TABLE 5.—Summarized data showing differences in host behavior with mosaic viruses of the common bean, pea 2, white clover, white sweetclover, alfalfa, red clover, and bean 3, on several hosts

Mosaic virus	Nature of symptoms on beans ¹		Number of bean varieties		Reaction on—							Seed transmission through beans artificially inoculated	
	Local	Systemic	Inoculated	Susceptible	<i>Phaseolus vulgaris</i>				<i>Pisum sativum</i> , Dwarf Telephone	<i>Lathyrus odoratus</i> , Baltimore Rose	<i>Melilotus alba</i> (white sweetclover)		
					Stringless Green Refugee	Corbett Refugee	Robust	Great Northern Idaho No. 1					
Common bean.....	—	—	31	28	+	—	—	—	—	—	—	—	+
Pea 2.....	—	—	31	30	—	—	—	—	—	—	—	—	—
White clover.....	—	—	31	29	—	—	—	—	—	—	—	—	—
White sweetclover.....	—	—	31	27	—	—	—	—	—	—	—	—	—
Alfalfa.....	—	—	31	26	—	—	—	—	—	—	—	—	—
Red clover.....	—	—	31	0	—	—	—	—	—	—	—	—	—
Bean 3.....	—	—	30	24	+	—	—	—	—	—	—	—	—

¹ Minus sign (—) indicates no infection; plus (+) indicates infection.

SEED TRANSMISSION

It has not been definitely proved that the viruses of any of the legumes except common bean mosaic are seed-borne, although preliminary evidence indicates that the virus may be carried in the seed of pea and white sweetclover in small percentages. Since the common bean mosaic is seed-borne, it was of interest to learn whether the viruses of other legumes, when inoculated to bean, would be so transmitted. Unfortunately, seed from beans infected with pea mosaic 2 and alfalfa mosaic was not collected, but that from plants infected with the mosaic disease of white clover and white sweetclover was. The seed of these plants was planted both in the greenhouse and in the field and allowed to grow to maturity, but in no case did any of the plants become diseased. Likewise, seed collected from *Vicia faba* plants infected with the viruses of these two clover mosaics as well as that of red clover mosaic produced healthy plants.

Larger populations of plants from seed from infected plants are necessary for testing before it can be definitely said that these mosaic diseases are not carried through the bean seed, but the evidence indicates that they are not.

PROPERTIES OF THE VIRUSES

Although the properties of the common bean mosaic were studied by Fajardo (7) and those of an alfalfa mosaic by Pierce (12), these studies were repeated in part in order to compare them with the properties of the viruses of pea mosaic 2, white clover, and white

sweetclover. The properties of the common bean mosaic, pea mosaic 2, and white sweetclover mosaic were based on the systemic lesions they produced on Stringless Green Refugee beans. The determinations of the white clover virus were based on the production of both systemic and local lesions on this same variety. The properties of the alfalfa virus were determined by local lesion production on the Red Valentine variety of beans.

The methods described by Johnson and Grant (9) were, in general, used for these determinations. In most cases 15 plants were inoculated except in some instances where 25 plants were used. The material for the thermal death-point studies was prepared by heating 2 cc of the expressed juice in thin glass tubes in a constant temperature bath at the desired temperature for 10 minutes and immediately cooling. The aging tests were conducted by placing the expressed juice in test tubes and allowing them to remain in the laboratory at room temperatures (75° to 80° F.) for the desired period. The dilution studies were made in the usual manner. The chemical tests were made by diluting the juice from diseased plants to the proper concentration of the chemical desired and allowing it to react for 30 minutes.

THEMAL DEATH POINT

The virus of the common bean mosaic was inactivated at about 58° C. (table 6), which confirms the results of Fajardo (7) and Pierce (12). The viruses of pea mosaic 2 and white sweetclover were also noninfectious at this temperature. The virus of white clover mosaic producing the systemic lesions on beans was inactivated at 58° to 60°, and the virus producing the local lesions at 62° to 65°. The alfalfa virus was inactivated at the same temperature, which agrees with the results of Pierce (12).

TOLERANCE TO DILUTION

The viruses of the common bean mosaic, pea mosaic 2, and white sweetclover mosaic were not infectious at dilutions greater than 1 to 1,000 (table 7). The virus of white clover mosaic producing the systemic and local lesions on beans and the alfalfa virus lost their infectivity at about 2 to 1,000 dilution.

LONGEVITY IN VITRO

The viruses of the common bean mosaic and white sweetclover mosaic were not infectious after aging in vitro from 28 to 32 hours (table 8). This is in agreement with Pierce (12) with respect to the common bean mosaic. The virus of pea mosaic 2 was inactivated at 24 to 28 hours. The white clover virus producing the systemic lesions on beans lost its infectivity after aging about 32 hours, whereas the virus producing the local lesions was inactivated at from 28 to 32 hours. The alfalfa virus was noninfectious at from 3 to 4 days' aging.

TABLE 6.—Comparison of thermal death point of viruses of common bean mosaic, pea mosaic 2, white clover mosaic, white sweetclover mosaic, and alfalfa mosaic, as determined by production of systemic and local lesions on beans

Reaction to virus of—															
Temperature (° C.)	Common bean mosaic		Pea mosaic 2		White clover mosaic					White sweet-clover mosaic		Alfalfa mosaic			
					Systemic infection		Local infection		Total lesions			Local infection		Total lesions	
	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected		Plants in-oculated	Plants infected	Plants in-oculated	Plants infected		Total lesions
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Not heated.....	15	12	15	12	15	12	15	15	3,715	15	15	15	15	15	1,347
54.....	15	10	15	13	15	10	-----	-----	-----	15	15	15	15	15	981
56.....	15	7	15	7	15	8	-----	-----	-----	15	6	15	15	15	601
58.....	15	0	15	0	15	9	15	15	3,692	15	0	15	15	15	298
60.....	-----	-----	-----	-----	15	0	15	15	3,180	-----	-----	15	13	15	105
62.....	-----	-----	-----	-----	-----	-----	15	15	2,718	-----	-----	15	10	26	-----
65.....	-----	-----	-----	-----	-----	-----	15	0	0	-----	-----	15	0	0	-----

¹ Total number of lesions produced on 30 inoculated primary leaves of Refugee Green bean variety.

² Total number of lesions produced on 30 inoculated primary leaves of Red Valentine bean variety.

TABLE 7.—Comparison of tolerance to dilution of viruses of common bean mosaic, pea mosaic 2, white clover mosaic, white sweetclover mosaic, and alfalfa mosaic, as determined by production of systemic and local lesions on beans

Dilution	Reaction to virus of—														
	Common bean mosaic		Pea mosaic 2		White clover mosaic					White sweet-clover mosaic		Alfalfa mosaic			
					Systemic infection		Local infection		Total lesions			Local infection		Total lesions	
					Plants in-oculated	Plants in-fected	Plants in-oculated	Plants in-fected				Plants in-oculated	Plants in-fected		
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
None.....	15	11	15	11	15	15	15	15	12,480	15	15	15	15	795	
1 to 10.....	15	11	15	14	15	15	15	15	-----	15	15	15	15	690	
1 to 100.....	15	8	15	8	15	15	-----	-----	-----	15	14	15	15	221	
1 to 200.....	15	4	15	5	15	14	-----	-----	-----	15	5	15	13	87	
1 to 400.....	25	2	25	3	25	13	15	13	35	25	4	15	9	28	
1 to 800.....	25	1	25	3	25	12	15	13	18	25	2	15	3	18	
1 to 1,000.....	25	0	25	0	25	6	15	13	13	25	0	15	4	12	
1 to 2,000.....	-----	-----	-----	-----	25	1	15	2	3	-----	-----	15	2	5	

¹ See footnote 1 to table 6.

² See footnote 2 to table 6.

TABLE 8.—Comparison of resistance to aging of viruses of common bean mosaic, pea mosaic 2, white clover mosaic, white sweetclover mosaic, and alfalfa mosaic, as determined by production of systemic and local lesions on beans

Time aged	Reaction to virus of—													
	Common bean mosaic		Pea mosaic 2		White clover mosaic					White sweet-clover mosaic		Alfalfa mosaic		
					Systemic infection		Local infection		Total lesions			Local infection		Total lesions
	Plants in-oculated	Plants in-fected	Plants in-oculated	Plants in-fected	Plants in-oculated	Plants in-fected	Plants in-oculated	Plants in-fected						
	No.	No.	No.	No.	No.	No.	No.	No.		No.	No.	No.	No.	
None	15	13	15	11	15	12	15	15	2,715	15	11	15	15	1,710
20 hours	15	11	15	4	15	7	15	11	54	15	8	15	15	
24 hours	15	4	15	3	15	8	15	11	54	15	7	15	15	
28 hours	15	1	15	0	15	4	15	3	24	15	5	15	15	
32 hours	15	0			15	2	15	0	0	15	0			
2 days					15	0						15	12	17
3 days												15	2	2
4 days												15	0	0

¹ See footnote 1 to table 6.

² See footnote 2 to table 6.

RESISTANCE TO CHEMICALS

The common bean mosaic virus was inactivated by 1 to 200 hydrochloric acid (35 to 37 percent) for 30 minutes (table 9). The viruses of pea mosaic 2, white sweetclover, alfalfa, and the virus of white clover producing the systemic lesions on beans were inactivated by 1 to 100 hydrochloric acid solution, while the virus of white clover producing the local lesions was still infectious at this concentration. The common bean mosaic virus lost its infectivity when treated with 50-percent alcohol. Pea virus 2, white clover mosaic virus, and white sweetclover mosaic virus were inactivated with 75-percent alcohol, whereas the alfalfa virus was still infectious at this concentration. The virus of common bean mosaic, that of white clover mosaic producing the systemic lesions on beans, and that of white sweetclover mosaic were not infectious when treated with a 1 to 500 solution of 37-percent formaldehyde. The virus of pea mosaic 2 was inactivated with a 1 to 1,000 dilution. The white clover virus producing the local lesions on beans and the alfalfa virus were inactivated with a 1 to 200 formaldehyde solution.

TABLE 9.—Comparison of resistance to chemicals of viruses of common bean mosaic, pea mosaic 2, white clover mosaic, white sweetclover mosaic, and alfalfa mosaic, as determined by production of systemic and local lesions on beans

Chemical ¹	Strength of chemical	Reaction to virus of—														
		Common bean mosaic	Pea mosaic ²	White clover mosaic							White sweet-clover mosaic	Alfalfa mosaic				
				Systemic infection		Local infection		Total lesions	Local infection	Total lesions						
				Plants inoculated	Plants infected	Plants inoculated	Plants infected					Plants inoculated	Plants infected	Plants inoculated	Plants infected	Total lesions
None.....		No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
Hydrochloric acid.....	1 to 500.....	15	12	15	11	15	13	15	15	2,490	15	14	15	15	15	3,282
Do.....	1 to 200.....	15	6	15	4	15	12	15	14	108	15	5	15	15	15	275
Do.....	1 to 100.....			15	0	15	2	15	7	7	15	6	15	4	4	0
Alcohol.....	25 percent.....	15	5	15	7	15	11	15	15	-----	15	8	15	15	15	3,228
Do.....	50 percent.....	15	0	15	4	15	7	15	15	742	15	4	15	15	15	568
Do.....	75 percent.....			15	0	15	0	15	0	0	15	0	15	14	14	120
Formaldehyde ⁴	1 to 2,000.....	15	10	15	7	15	6			-----	15	3				-----
Do.....	1 to 1,000.....	15	4	15	0	15	6			-----	15	1	15	15	15	2,610
Do.....	1 to 500.....	15	0			15	0	15	15	139	15	0	15	15	15	1,902
Do.....	1 to 200.....							15	0	0			15	0	0	0

¹ 30-minute treatments.

² See footnote 1 to table 6.

³ See footnote 2 to table 6.

⁴ Concentrated solution containing 37-percent formaldehyde.

DISCUSSION

The evidence presented in this paper shows that the viruses of certain legume mosaic diseases can be transmitted to beans, and that under certain conditions, especially in the field, the symptoms of some may be mistaken for the common bean mosaic. Under controlled conditions in the greenhouse little difficulty is experienced in distinguishing the various diseases when the viruses are inoculated to bean. The more susceptible varieties, such as Stringless Green Refugee, are especially suitable for such diagnostic purposes. Furthermore, there is a definite varietal behavior to the several viruses which serves as a further means of differentiating between them.

It has been demonstrated by the senior writer (23) that several species of aphids are capable of transmitting the common bean mosaic virus, and it is probable that they are responsible for most of the secondary spread of the disease in the field. That some of this secondary spread may be caused by aphids transmitting certain of the legume mosaic viruses to bean is entirely probable. It seems likely that aphids feeding on mosaic-diseased legumes growing in close proximity to beans may transmit the virus to bean. The extent of this spread is not known, but in some cases it might be considerable. It may tend to explain why under certain conditions a field containing an extremely small amount of primary mosaic infection in the early part of the growing season may at the end be very severely infected.

Field examinations in many sections of the country have shown that the mosaic diseases of some of the clovers are probably present wherever the hosts are found. In many of the bean-growing sections of the United States the sweetclovers are not only grown as cultivated crops but are also found growing in abundance along the highways, irrigation ditches, and fence rows.

Some of the clovers belonging to the genus *Trifolium* are found almost everywhere in the United States where beans are grown. No systematic search has been made for the occurrence of the mosaic diseases of the legumes already discussed; however, some were found without difficulty wherever searches were made.

The symptoms of the mosaic-infected legumes are more easily recognized in the spring and in early summer than later in the season. In the case of peas grown at high altitudes, the mosaic symptoms are best recognized later in the summer. In general the diseases do not appear to stunt the plant, except possibly pea and bean, and then only when the infection occurs early in the development of the plant. Severely infected white and yellow sweetclover plants may be stunted in the spring, but later in the season they appear to be almost as vigorous as normal plants.

Pea mosaic is not so wide-spread as common bean mosaic. Pea mosaic virus 1 has been found by the writers in Maryland, Montana, Washington, and California. Linford⁶ in a survey of pea diseases, found mosaic in nine States, extending from the Atlantic coast west to Utah and Montana. He stated that in Maryland and New Jersey it reached its maximum in both abundance and severity. Pea mosaic virus 2 has as yet been found only in Maryland and Colorado, and it is believed to be less wide-spread than the common pea mosaic virus. In the canning sections of the Eastern and Middle Western States peas are usually removed from the land before the beans in the vicinity have grown to appreciable size, and hence the danger of spread from peas infected with virus 2 to beans is remote except where crop sanitation is not practiced. In the seed and market garden growing areas of the western United States peas are seldom grown in the same localities where beans are produced, which eliminates the probability of mosaic infection from that source.

White sweetclover mosaic is found abundantly in the District of Columbia and in the contiguous sections of Maryland and Virginia. It is probably equally as wide-spread in other parts of these two States. It has also been found in Wisconsin, Iowa, Nebraska, and Colorado. Surveys made in other States most likely would reveal the occurrence of the disease in them.

White clover mosaic is possibly as wide-spread as white sweetclover mosaic, but because the symptoms are somewhat inconspicuous it may be easily overlooked. It has been found wherever sought, but since white clover is not commonly grown in the Western States, it is of little importance there, and would not be a factor in the spread of its mosaic to beans. In the eastern and middle western bean-growing sections it may be a more important factor.

Alfalfa mosaic does not appear to be as wide-spread as the virus diseases of white clover and white sweetclover. A careful search has been made in the East and in some of the intermountain States,

⁶ LINFORD, M. B. PEA DISEASES IN THE UNITED STATES IN 1923. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Repr. Sup. 67: 1-14. 1929. [Mimeographed.]

but only a small amount was found. Under high temperatures the symptoms are masked, and since the inspections were made in the summer it is likely that it was present but its symptoms were masked. Under California conditions, Weimer (20) reports it as being especially abundant in the spring of the year before the first cutting.

In the spring of 1931 white sweetclover mosaic was found very abundantly in the vicinity of Rosslyn, Va., and marked symptoms were noted on practically all second-year growth. Preliminary evidence indicates that this mosaic is not seed-borne, but if so it is only in small amounts. The virus apparently overwinters in the roots of the plant and appears in the first growth in the spring. Aphids are commonly found feeding on such plants and probably transfer the virus to seedlings.

The rapidity and ease with which white sweetclover mosaic may be transmitted from plant to plant was demonstrated under greenhouse conditions. In the fall of 1932 about 200 healthy seedlings were planted in a greenhouse bench. The house was fumigated regularly until May, at which time most of the experimental work was terminated. At this time about 5 percent of the plants were inoculated and produced typical symptoms. The plants were allowed to grow during the summer, but no precaution was taken to control aphids. In the fall of 1933 all the plants were infected, the disease probably having been spread by the aphids feeding on the plants during the summer. It is assumed that the disease is spread in this manner under field conditions from the biennial plant to the seedlings and hence is present every year.

White clover mosaic does not appear to be spread as readily as white sweetclover mosaic, since initially infected areas do not enlarge very rapidly. Aphids are found on this clover, but seldom in large numbers. The virus appears to overwinter in the roots as it does in white sweetclover, and reappears in the new growth in the spring.

It has already been pointed out that red clover mosaic is not transmissible to beans, although it was shown by Doolittle and Jones (4), and substantiated by the writers, that it is transmissible to peas and sweet peas. Doolittle and Jones stated that it seems probable that red clover acts as a source of mosaic infection to peas in the field.

It is quite likely that pea mosaic virus 1, which is not infectious to beans, may have been the same as the one reported by Doolittle and Jones (4) and may be identical with red clover mosaic. The fact that pea mosaic virus 2 was infectious to beans clearly shows that there are at least two forms of mosaic found on peas, and further studies may demonstrate more.

The symptoms of red clover mosaic are not all alike. In fact, they may be so variable as to indicate the possibility of more than one form. Preliminary studies on peas have indicated that there may be two forms, but a more detailed study of the viruses is necessary before any definite conclusions can be drawn. Whether all forms of pea mosaic are actually identical with one or the other of the clover mosaic diseases transmitted to peas is still unknown. Doolittle and Jones (4) were unsuccessful in transmitting the mosaics of sweetclover and bean to garden peas and sweet peas. The writers, on the other hand, transmitted the mosaic diseases of white sweetclover and white clover to pea and sweet pea. The symptoms pro-

duced on pea by these two viruses were indistinguishable from those caused by the pea viruses 1 and 2. They can be identified, however, by means of a cross inoculation to beans, where symptoms identical to those produced by the viruses of the specific hosts are produced.

Results indicate that there may be more than one form of white clover mosaic, as shown by inoculation studies with beans and peas. White clover mosaic was obtained from several sources. Two specimens collected in the Eastern States gave identical results in that both produced local and systemic infection as previously described. Specimens collected in Colorado produced no local lesions, and the systemic lesions on Stringless Green Refugee beans somewhat resembled the symptoms produced by pea mosaic virus 2 except that the infection was not so severe.

The production of local and systemic lesions on beans by white clover mosaic suggests the possibility of two distinct viruses, one producing local and the other systemic lesions. Numerous sources of white clover mosaic were used in the experimental work, with similar results.

Since many of the clover mosaic viruses can be transmitted to other leguminous hosts, it is likely that infection studies alone might be misleading. The study of symptoms on susceptible bean varieties, and of the reaction to these viruses of the varieties resistant to the common bean mosaic, is helpful in separating them. That more than one virus may infect a single host is possible. Since white sweetclover is susceptible to white clover mosaic, it might harbor both viruses at the same time. Two or more viruses combined on one host might produce symptoms on beans different from those reported here. From this it is apparent that symptomatology is not alone sufficient in the diagnosis of virus diseases of many of the legumes, but that a study of the particular virus as to host reaction and properties becomes essential.

There have been a number of reports showing that in addition to the common bean mosaic other viruses are capable of being transmitted to bean. Carsner (2) showed that the virus of curly top of sugar beets is transmissible to beans. Nelson's rugose mosaic of beans (11) appears to be different from any of the virus diseases reported here, especially on the basis of seed transmission. Pierce's yellow bean mosaic (12), on the basis of symptoms, transmission, and properties of the virus, appears to be a form of white sweetclover mosaic described previously by the writers (24) and not truly a bean mosaic. It is evident from a comparison of varietal resistance and susceptibility of beans that, although yellow bean mosaic is somewhat similar to the white sweetclover mosaic described here, it is not identical therewith. Pierce (12) reports that no varieties of the common bean have been found which are entirely resistant to the yellow bean mosaic. It is possible that these differential reactions may be due to the various bean strains employed.

Data presented here show that Great Northern Idaho No. 1, Robust, California White, and Red Kidney (Dark Mahogany strain) beans are resistant to the white sweetclover mosaic virus. Pierce reported no test on the last two varieties, but found the Great Northern Idaho No. 1 and Robust varieties susceptible to yellow

bean mosaic. Yellow bean mosaic was reported to be noninfectious to peas (Perfection variety). The writers, however, found the white sweetclover mosaic to be readily transmitted to this variety.

Merkel (10) in his transmission work with aphids concluded that the same virus was responsible for the mosaic of *Phaseolus vulgaris*, *Pisum sativum*, *Lathyrus odoratus*, *Lupinus luteus*, *Melilotus altissima*, *Trifolium pratense*, *T. hybridum*, *T. repens*, *Anthyllis vulneraria*, and *Vicia faba*. It is apparent from the writers' data that more than a single virus is responsible for the mosaic diseases of these legumes.

Elliott's (5) mosaic virus of sweetclover and red clover was shown by him to be mutually transmissible as well as infectious to *Medicago arabica* and *Vicia faba*. He was unable, however, to infect *Trifolium repens* with it. This virus does not appear to be the same as that causing the mosaic disease of white sweetclover and red clover reported here, because of the inability of these two viruses to be cross-inoculated. The virus of red clover has been shown by Dickson (3) to be transmissible to a number of species of the genus *Trifolium* and to *Medicago lupulina*, but not to *Melilotus alba* or to *M. officinalis*. This mosaic may be similar to the red clover mosaic reported here but different from the pea mosaic virus 2, which, as has been pointed out, is not identical with the pea mosaic described by Doolittle and Jones (4). Böning's virus disease from broadbean (1), which he was able to transmit to red clover, crimson clover, and peas, may be identical with the pea mosaic virus 1 and the red clover mosaic reported by the writers. It may also be identical with the pea mosaic of Doolittle and Jones (4). Pierce (12) states that the alfalfa mosaic described by him was distinct from the mosaic studied by Weimer (19, 20) and later by the writers (22, 24). The differences were not pointed out. Weimer's alfalfa mosaic differs from the one discussed by Pierce (12) in its reaction to Corbett Refugee bean and to peas. Pierce states that Corbett Refugee is slightly susceptible to his alfalfa mosaic, whereas the writers were unable to infect this variety. He also reports peas as being susceptible, while negative results were secured with the alfalfa mosaic used in the studies reported here. Since these differences are slight, it is not unlikely that Pierce's alfalfa mosaic is identical with Weimer's, as reported earlier by the writers (22, 24) as infecting beans.

Henderson's ring spot of sweetclover (8), which he transmitted to Turkish tobacco and petunia, is distinct from the white sweetclover mosaic reported here. The symptoms of this disease on white sweetclover differ from those of the mosaic disease of this host.

As yet little is known regarding the economic importance of the spread of the legume mosaics to bean. It is believed that they are responsible for considerable losses. Because of the similarity, under certain conditions, of symptoms between the common bean mosaic and certain of the legume mosaics on bean, it is not unlikely that they have been mistaken for bean mosaic. Since the mosaic viruses of white clover and white sweetclover on bean have not been proved thus far to be seed-borne, it appears that they will never reach the importance of the common bean mosaic. The location of the bean plot so as not to be in close proximity to cultivated legumes, especially the clovers, and the eradication of those growing wild, may be helpful

in controlling, at least in part, the secondary spread of these virus diseases to beans.

The development by selection of varieties resistant to the common bean mosaic (Corbett Refugee, Great Northern Idaho No. 1, and Robust) has been accomplished. These varieties have been used as parental stock in the production of other varieties of the canning and market-garden types resistant to the common bean mosaic. It is possible that varieties resistant to all of the legume mosaic diseases can be developed. The facts that Great Northern Idaho No. 1 is resistant to most of these diseases and Corbett Refugee and Robust are resistant to others offer promising parent material for the development and improvement of other resistant varieties.

SUMMARY

The viruses causing the mosaic disease of pea, white clover, alsike clover, white sweetclover, alfalfa, and sweet pea are all transmissible to beans, while the virus of red clover mosaic is not. The mosaic virus obtained from a bean hybrid proved to be distinct from the common bean mosaic and from any of the clover mosaics.

The symptoms of the mosaic viruses from white clover, alsike clover, and white and yellow sweetclover, when inoculated to bean, may under certain conditions resemble the mottled and chlorotic conditions of the common bean mosaic. However, a careful examination under greenhouse conditions reveals specific differences.

It is probable that various other mosaic viruses present in the several legumes may react differently from those reported in this paper notwithstanding similarity of symptoms.

The legume mosaic viruses produced systemic infection on beans, with the exception of alfalfa virus, which produced only local lesions. The white clover mosaic virus may produce both systemic and local lesions.

The susceptibility and resistance of 31 bean varieties to the viruses of the legumes mentioned were determined. Thirty out of 31 varieties of beans tested were susceptible to the pea mosaic virus 2, and the white clover mosaic virus produced typical symptoms on 29 out of 31 varieties inoculated. The white clover mosaic virus on beans produced local lesions on 26 out of 30 varieties tested. White sweetclover mosaic infected 27 out of 31 varieties. Twenty-nine out of 31 varieties of beans were susceptible to the alfalfa mosaic virus. The virus of bean mosaic 3 infected 24 out of 30 varieties tested.

The three bean varieties resistant to the common bean mosaic do not react identically when inoculated with the viruses of the various legumes. Great Northern Idaho No. 1 is resistant to all of the viruses used except to that of the alfalfa mosaic. Robust is susceptible to the viruses of pea mosaic 2, white clover mosaic, and alfalfa mosaic. Corbett Refugee is resistant only to the alfalfa virus. Bean mosaic virus 3 is infectious only to Corbett Refugee.

The local lesions produced by tobacco mosaic and the tobacco ring spot viruses are different from those produced by the mosaic viruses of white clover and alfalfa, both in symptoms and in varietal susceptibility.

The various leguminous hosts showed differences in resistance and susceptibility when inoculated with the several legume mosaic viruses.

No evidence of transmission of white clover mosaic, white sweetclover mosaic, and bean mosaic virus 3 through bean seed was obtained.

The thermal death point, tolerance to dilution, resistance to aging in vitro, and resistance to chemicals were determined for all the viruses. The thermal death point of the viruses of the common bean mosaic, pea mosaic 2, and white sweetclover mosaic was found to be between 56° and 58° C. The virus of white clover mosaic producing the systemic lesions on beans was inactivated between 58° and 60°, while that producing the local lesions on beans and also the alfalfa virus lost their infectivity at 62° to 65°. As for tolerance to dilution, the viruses of the common bean mosaic, pea mosaic 2, and white sweetclover mosaic were not infectious at dilutions greater than 1 to 1,000. The viruses of white clover mosaic producing both the systemic and local lesions on beans and the alfalfa virus were still infectious at 1 to 2,000 dilution. In experiments on aging in vitro, the virus of the common bean mosaic and white sweetclover mosaic were no longer infectious after 28 to 32 hours; the virus of pea mosaic 2 lost its infectivity at from 24 to 28 hours; that of white clover producing the local lesions on beans was inactivated when aged from 28 to 32 hours, whereas the virus producing the systemic lesions was not infectious after 32 to 48 hours. The alfalfa virus was inactivated after aging for 3 to 4 days. With respect to resistance to chemicals there were certain differences between the various dilutions of hydrochloric acid, alcohol, and formaldehyde.

Pea mosaic virus 2 is not believed to be as wide-spread as the common pea mosaic. Alfalfa mosaic appears to be limited in its dissemination. The mosaic diseases of white clover and white sweetclover have been found in many localities, and it is assumed that they are present wherever the hosts are grown. It is possible that beans may become infected from the various legume mosaic viruses in the field as a result of aphid transmission. Until varieties resistant to these diseases have been developed, bean fields should be located as far from infected commercial clover plantings as possible. The elimination of legumes growing wild along roadways, fence rows, and irrigation ditches may prove an effective control measure.

LITERATURE CITED

- (1) BÖNING, K.
1927. DIE MOSAIKKRANKHEIT DER ACKERBOHNE (*VICIA FABA* L.). EIN BEITRAG ZU DEM MOSAIK DER PAPILIONACEEN. *Forschungen Gebiet Pflanzenkrank. u. Immunität Pflanzenreich* 4: [43]-111, illus.
- (2) CARSENER, E.
1926. SUSCEPTIBILITY OF THE BEAN TO THE VIRUS OF SUGAR-BEET CURLY-TOP. *Jour. Agr. Research* 33: 345-348, illus.
- (3) DICKSON, B. F.
1922. STUDIES CONCERNING MOSAIC DISEASES. *MacDonald Col. Tech. Bull.* 2, 125 pp., illus.
- (4) DOOLITTLE, S. P., and JONES, F. R.
1925. THE MOSAIC DISEASE IN THE GARDEN PEA AND OTHER LEGUMES. *Phytopathology* 15: [763]-772, illus.
- (5) ELLIOTT, J. A.
1921. A MOSAIC OF SWEET AND RED CLOVERS. *Phytopathology* 11: 146-148, illus.

- (6) FAJARDO, T. G.
1930. STUDIES ON THE MOSAIC DISEASE OF THE BEAN (*PHASEOLUS VULGARIS* L.). *Phytopathology* 20: 469-494, illus.
- (7) ———
1930. STUDIES ON THE PROPERTIES OF THE BEAN-MOSAIC VIRUS. *Phytopathology* 20: 883-888.
- (8) HENDERSON, R. G.
1934. OCCURRENCE OF TOBACCO RING-SPOT-LIKE VIRUSES IN SWEET CLOVER. *Phytopathology* 24: 248-256, illus.
- (9) JOHNSON, J., and GRANT, T. J.
1932. THE PROPERTIES OF PLANT VIRUSES FROM DIFFERENT HOST SPECIES. *Phytopathology* 22: 741-757.
- (10) MERKEL, L.
1929. BEITRÄGE ZUR KENNTNIS DER MOSAIKKRANKHEIT DER FAMILIE DER PAPILIONACEEN. *Ztschr. Pflanzenkrankh.* 39: [289]-347, illus.
- (11) NELSON, R.
1932. INVESTIGATIONS IN THE MOSAIC DISEASE OF BEAN *PHASEOLUS VULGARIS* L.). *Mich. Agr. Expt. Sta. Tech. Bull.* 118, 71 pp., illus.
- (12) PIERCE, W. H.
1934. VIROSES OF THE BEAN. *Phytopathology* 24: 87-115, illus.
- (13) ——— and HUNGERFORD, C. W.
1929. SYMPTOMATOLOGY, TRANSMISSION, INFECTION, AND CONTROL OF BEAN MOSAIC IN IDAHO. *Idaho Agr. Expt. Sta. Research Bull.* 7, 37 pp., illus.
- (14) PRICE, W. C.
1930. LOCAL LESIONS ON BEAN LEAVES INOCULATED WITH TOBACCO MOSAIC VIRUS. *Amer. Jour. Bot.* 17: 694-702, illus.
- (15) REDDICK, D., and STEWART, V. B.
1918. VARIETIES OF BEANS SUSCEPTIBLE TO MOSAIC. *Phytopathology* 8: [530]-534.
- (16) ——— and STEWART, V. B.
1919. ADDITIONAL VARIETIES OF BEANS SUSCEPTIBLE TO MOSAIC. *Phytopathology* 9: [149]-152.
- (17) ——— and STEWART, V. B.
1919. TRANSMISSION OF THE VIRUS OF BEAN MOSAIC IN SEED AND OBSERVATIONS ON THERMAL DEATH-POINT OF SEED AND VIRUS. *Phytopathology* 9: [445]-450.
- (18) STEWART, V. B., and REDDICK, D.
1917. BEAN MOSAIC. (Abstract) *Phytopathology* 7: 61.
- (19) WEIMER, J. L.
1931. ALFALFA MOSAIC. (Abstract) *Phytopathology* 21: 122-123.
- (20) ———
1934. STUDIES ON ALFALFA MOSAIC. *Phytopathology* 24: 239-247, illus.
- (21) WINGARD, S. A.
1928. HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS. *Jour. Agr. Research* 37: 127-153, illus.
- (22) ZAUMEYER, W. J.
1933. TRANSMISSIBILITY OF CERTAIN LEGUME MOSAIC VIRUSES TO BEAN. (Abstract) *Phytopathology* 23: 39.
- (23) ———
1933. TRANSMISSION OF BEAN-MOSAIC VIRUS BY INSECTS. (Abstract) *Phytopathology* 23: 40.
- (24) ——— and WADE, B. L.
1933. MOSAIC DISEASES AFFECTING DIFFERENT LEGUMES IN RELATION TO BEANS AND PEAS. (Phytopathological note) *Phytopathology* 23: 562-564.

RANDOM SAMPLING AND THE DISTRIBUTION OF PHENOTYPES ON EARS OF BACKCROSSED MAIZE¹

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INTRODUCTION

The distribution of a given phenotype on ears of maize (*Zea mays* L.) segregating for an endosperm character should be purely random if determined by chance alone. In classifying kernels on backcrossed ears the writer gained the definite impression that the distribution was not random. In conversation with other investigators he found that some held a similar opinion and that others thought the distributions were quite random. The data presented here were collected to test the randomness of distributions of two contrasting phenotypes.

MATERIAL AND METHODS

Maize plants of the constitution *Y-y Su-su* were pollinated with *y-su* pollen. This permits the expression of the gametophyte as determined at meiosis in the pistillate inflorescence. The cross made in this way also permits a more accurate separation of yellow (*Y*) and white (*y*) seeds and eliminates any possible disturbing effect of pollen-tube growth factors or differential establishment of pollen tubes.

The phenotype of each kernel for each of 81 ears from this cross was recorded by code, as illustrated in figure 1. The frequencies of groups of 1, 2, 3, etc., individuals were then tabulated. It was originally planned to study the distribution of phenotypes both vertically and laterally on the ear. During the accumulation of the data, however, it became apparent that distribution in the lateral direction might be inaccurate because of spatial changes occurring between meiosis and maturity. The data presented represent the distributions in vertical rows only.

EXPERIMENTAL RESULTS

The contrasting phenotypes, *Y-y* and *Su-su*, are expected to occur with equal frequency. With random distribution the expected number of groups of a stated size will be given by the expression $(.5^n)N$, where n is the number of kernels in the group in question and N is the total number of groups.

Two methods have been used in estimating the randomness of the various distributions. The first estimate used was P , calculated from the χ^2 test. The second method² attempts to answer the question whether or not the data are consistent with an independent 1:1 chance for each kernel. If there are a individuals in a set of consecutive kernels, the phenotypic change from one kernel to the next is independently decided $a-1$ times. The number of possible changes in any given population is then $S(a-1)$. If this value differs significantly from half that of $S(a)$, then an independent 1:1 chance for

¹ Received for publication July 24, 1935; issued December 1935.

² The writer is indebted to R. A. Fisher for suggesting this procedure.

TIP

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// Y Su.....Yellow Starchy
 /- Y su.....Yellow Sugary
 -/ y Su.....White Starchy
 -- y su.....White Sugary
 0.....Missing Kernel

BOTTOM

FIGURE 1.—Method of recording the phenotypic constitution of individual kernels on a backcrossed ear of corn.

each kernel has not been realized. The deviations of $\frac{1}{2}S(a) - S(a-1)$ divided by the probable error furnish the second estimating statistic. Hereafter the first method will be designated as the probability method and the second the deviation method.

The observed frequencies for the individual phenotypic classes are presented in table 1. It is quite apparent that the distributions are not random in any case. The distributions of the sum of the yellow-white and starchy-sugary group also are presented. This distribution is merely one of groups of a given size irrespective of phenotype. In both the yellow-white and starchy-sugary combinations the χ^2 values are higher than those of either component distribution. This indicates that the distributions tend to be biased in the same direction. It is apparent that this bias operates to increase the number of groups with one kernel per group and decrease the number of groups with three or more kernels.

TABLE 1.—Frequencies of phenotypic groups of a given size, and probability of randomness of these frequencies

Kernels per group (number)	Frequencies of groups of phenotype indicated					
	Y	y	Y+y	Su	su	Su+su
1.	2,522	2,572	5,094	2,532	2,639	5,171
2.	1,159	1,155	2,314	1,213	1,148	2,361
3.	595	553	1,148	536	522	1,058
4.	256	265	521	265	255	520
5.	124	111	235	126	117	243
6.	67	51	118	50	67	117
7.	25	24	49	34	33	67
8.	13	17	30	11	11	22
9+.	9	14	23	12	11	23
Total	4,770	4,762	9,532	4,779	4,803	9,582
χ^2	30.45	45.89	70.81	36.51	57.96	87.88
P	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002

Missing kernels should occur in the different classes in proportion to the total number of kernels in each class. Twenty-five percent therefore should occur in classes of one individual, 25 percent in classes of two individuals, etc. Missing kernels in the first class will result in an increase in group size because of combining adjacent groups. Groups originally involving two or more kernels but depleted by a single missing kernel will contribute equally to all groups smaller than the one in which the miss occurs. The occurrence of groups of missing kernels, which is noted much more rarely, likewise contributes to the smaller groups, though the contributions may not be equal. The net effect is an increase in the number of one-kernel groups and a decrease of all other size groups.

The distribution of groups adjacent to a missing kernel was tabulated and found to be in general agreement with the expectations outlined above. This indicates that missing kernels is one factor contributing to the bias indicated in table 1, and in consequence correction for missing kernels appears legitimate.

Missing kernels may be corrected for in two ways, by eliminating all kernel rows with missing kernels and by eliminating the groups adjacent to a missing kernel. A priori it would appear that these two methods should be equally effective. The comparative results are presented in table 2.

TABLE 2.—*Estimates of randomness of the frequencies of phenotypic groups after correction is made for missing kernels*

Source of data	Estimating statistic	Values for phenotype indicated					
		Y	y	Y+y	Su	su	Su+su
Entire data; no correction.....	P^1	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Corrected for missing kernels.....	D^1	7.92	10.25	12.65	8.9	10.6	13.8
Elimination of groups adjacent to skip ¹	$\{P$29	.25	.04	.07	.06	.01
.....	D	2.3	4.1	4.5	4.2	5.4	6.5
Elimination of kernel rows containing a skip ²	$\{P$38	.35	.11	.15	.04	.02
.....	D	2.0	3.0	3.5	3.4	5.4	6.2

¹ Determined from χ^2 .² The deviation of $\frac{1}{2}S(a) - S(a-1)$ divided by its probable error.

When the correction for missing kernels is accomplished by eliminating groups adjacent to the skip (table 2, method 1), the distribution for each phenotype is within the limits of random sampling as estimated by P determined from χ^2 . The fits for the starchy and for the sugary distributions are considerably poorer than those for the yellow and white. In both cases the distribution of the sums gives a poorer fit than either component, indicating that a slight bias still exists.

When the correction for missing kernels is accomplished by eliminating kernel rows with missing kernels (table 2, method 2), the probability for the randomness of all distributions, save one, is slightly higher than when the previous method of correction is used. The differences between the two methods, however, are not significant.

The corrected data indicate that for the starchy-sugary distributions a significant bias still is operative. There is a suggestion that a smaller though similar bias is operative in the yellow-white distributions. The deviations on the basis of an independent 1:1 chance for each kernel are clearly significant.

In the maize ear, judging from the development of the silks, meiosis begins in a region near the base of the ear and proceeds in a regular manner in both directions. The distribution of the end group of each kernel row might conceivably be biased, owing to a possible limitation in size. The possibility was tested by separating the population into two subgroups. One subgroup was composed of the end group of each kernel row, and the other was composed of the remainder of the ear. The results are presented in table 3. The subgroup composed of the end group of the butt and tip of each kernel row is quite random, as indicated by both the probability and the deviation methods. The subgroup composed of the remainder of the ear is clearly not random. The distributions of these two subgroups for the total $Y-y$ and $Su-su$ phenotypes are compared with theoretical expectancy in figures 2 and 3.

TABLE 3.—*Estimates of randomness of the frequencies of phenotypic groups after separation is made into butt-tip and middle subgroups*

Source of data	Estimating statistic	Values for phenotype indicated					
		Y	y	Y+y	Su	su	Su+su
Subgroups:							
Butt-tip.....	$\{P^1$	0.49	0.26	0.28	0.76	0.80	0.47
.....	D^11	.8	.6	1.7	.3	.4
Remainder.....	$\{P$61	.09	.02	.04	.002	.001
.....	D	2.8	5.2	5.4	4.1	6.1	7.0

¹ Determined from χ^2 .² The deviation of $\frac{1}{4}S(a) - S(a-1)$ divided by its probable error.

A bias which involves all portions of the ear except the end groups might arise through limitations imposed by the length of each kernel row. As an extreme example, a group of 8 kernels could have its point of origin in only three places in a row having only 10 kernels, and only one of these could be included in the subgroup designated as "remainder" in the preceding paragraph. In other words, only 10 percent of the theoretical places of origin can lead to an inner group of eight kernels. A similar but decreasingly important limitation will be noted as longer and longer rows are involved. An estimate of the importance of this limitation was obtained by selecting two subgroups from the total population. One group was composed of ears

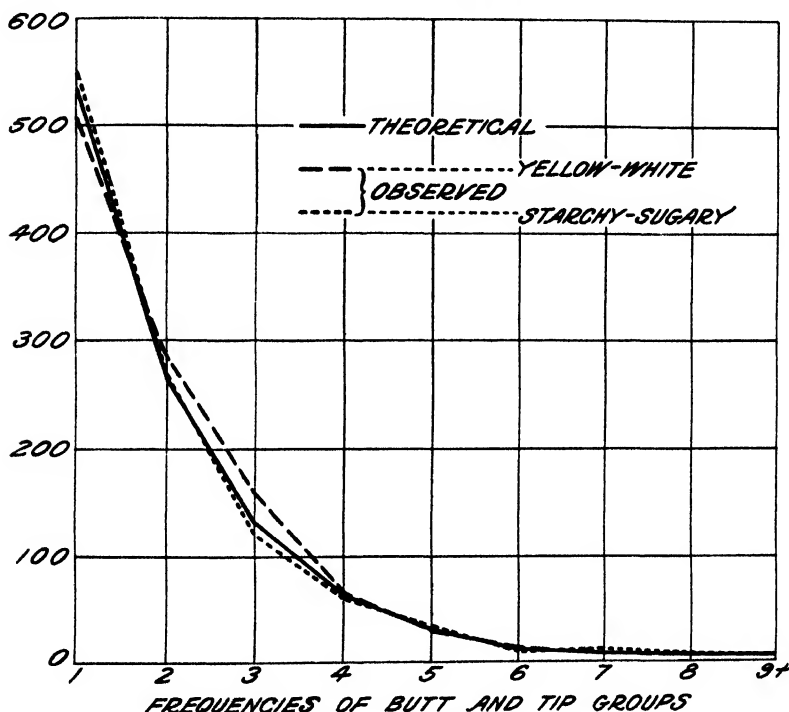


FIGURE 2.—Comparison of observed and theoretical distributions in the subgroup composed of the butt and tip end groups.

having 25 or fewer kernels per row with a mean of 19. The contrasting group had 35 or more kernels per row with a mean of 38. The comparisons are presented in table 4.

TABLE 4.—Estimates of randomness of the frequencies of phenotypic groups after separation is made into long and short kernel row subgroups

Source of data	Estimating statistic	Values for phenotype indicated					
		Y'	y	Y+y	Su	su	Su+su
Subgroups:							
35 or more kernels per row.	$\{P$	0.06	0.13	0.12	0.50	0.04	0.76
	$\{D$	2.80	2.13	2.92	.37	2.57	2.05
25 or fewer kernels per row	$\{P$.06	.14	.08	7.01	.30	.01
	$\{D$	3.77	2.78	4.64	6.44	1.72	5.89

¹ Determined from χ^2 .

² The deviation of $\frac{1}{2}S(a) - S(a-1)$ divided by its probable error.

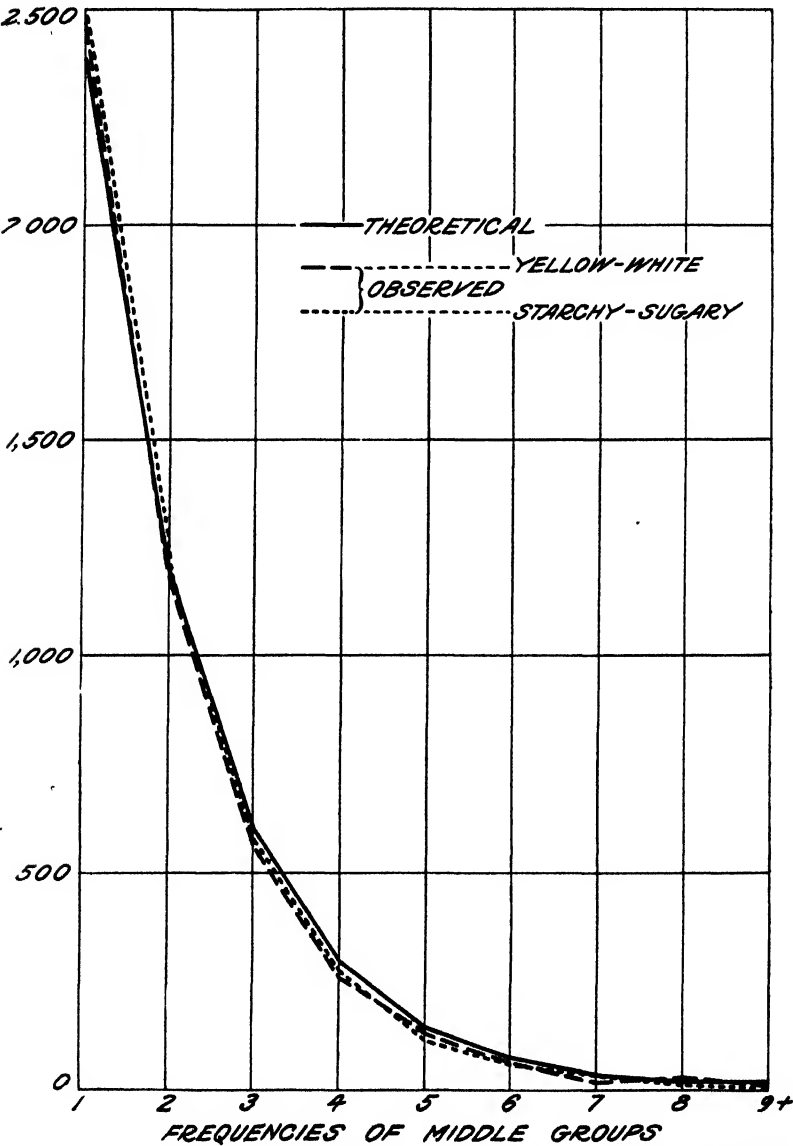


FIGURE 3.-Comparison of observed and theoretical distributions in the subgroup composed of all the ear except the butt and tip end groups

In the majority of cases the distributions for the ears with longer kernel rows are more nearly in accord with expectancy than for the shorter, though the differences are not great. By a process of combining adjacent end groups when similar in phenotype, it is possible to consider the material from the 81 ears as belonging to a single kernel row and thus eliminate variations due to length of row from ear to ear. This has been done, and the comparison is presented in table 5.

TABLE 5.—*Estimates of randomness of the frequencies of phenotypic groups when the entire population is treated as a single kernel row*

Source of data	Estimating statistic	Values for phenotype indicated					
		<i>Y</i>	<i>y</i>	<i>Y+y</i>	<i>Su</i>	<i>su</i>	<i>Su+su</i>
All ears combined in 1 kernel row	$\{P^1$	0.11	0.77	0.10	0.36	0.72	0.95
	$\{D^1$	1.22	.12	.95	.28	1.31	1.29

¹ Determined from χ^2

² The deviation of $\frac{1}{2} S(a) - S(a-1)$ divided by its probable error.

This distribution was also corrected for missing kernels by disregarding groups adjacent to a missing kernel. When this comparison is made the distribution is entirely random as judged by either estimating statistic. This distribution for the *Y-y* segregation is given in figure 4.

The segregation for the contrasting allelomorphs *Y-y* and *Su-su* are in fair agreement with the expected 1:1 distribution as judged by their deviation-probable error values, but there is a slight excess over expectancy of ears having large deviations. A comparison of the relative agreement with expectancy for ears of this group with ears whose deviations are half their probable error or less showed no significant difference between these two subgroups.

The correlation between size of adjacent groups was determined from a double-entry table. In the preparation of this table, butt and tip groups and groups adjacent to a missing kernel were not used. Thus the first and last usable groups of each kernel row were involved in only one comparison, while the intervening groups were used twice. The values of *r* so calculated were found to be -0.001 ± 0.008 for the *Y-y* and -0.004 ± 0.0123 for the *Su-su* phenotypes. Neither of these values differs significantly from zero, which is the value expected with a purely random distribution.

SUMMARY

The distribution of groups of 1, 2, 3, etc., kernels of the yellow-white and starchy-sugary phenotypes was studied on 81 back crossed ears of maize. The data as originally collected were found not to be random, contrary to expectation.

A bias operated to increase the number of groups with 1 kernel per group and to reduce the number having 3 or more.

Missing kernels and length of kernel row were found to be the chief sources of this bias. When corrections were made for these disturbances, the distributions were well in accord with expectancy.



FIGURE 4.—Distribution of the Y-y phenotypes from 81 ears considered as being from a single kernel row. Starting at the top of the left column, the distribution proceeds down each column progressively toward the right. The classes marked with an asterisk represent combined classes at the junction between two ears. A horizontal dash between classes indicates a junction between ears in which the end groups were unlike and therefore not combined.

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INTERGENERIC HYBRIDS OF TRITICUM AND SECALE WITH HAYNALDIA VILLOSA¹

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INTRODUCTION

In recent years, particularly during the last decade, considerable effort has been directed by various investigators to the hybridization of the genus *Triticum* with the closely related genera *Aegilops*, *Secale*, *Haynaldia*, and *Agropyron*. This has been done to extend the knowledge on their genetics and cytology, to supply possible information on the phylogenesis of *Triticum*, and to determine further the economic possibilities of intergeneric and interspecific hybridization.

The early literature involving hybridizations of *Triticum*, *Aegilops*, and *Secale* has been reviewed by Bleier (1),² Kajanus (3), Watkins (12), Longley and Sando (4), and others. More recently Oehler (5) has published an extensive paper on crosses of *Triticum* with *Aegilops*. Of the 5 genera, i. e., *Triticum*, *Aegilops*, *Secale*, *Haynaldia*, and *Agropyron*, the last 2 have received less attention by investigators.

Haynaldia has been classified botanically under *Triticum*, *Secale*, and *Agropyron* and as an independent genus. The writer considers, however, that *Haynaldia* is differentiated from *Triticum*, *Secale*, *Aegilops*, and *Agropyron* by characters of sufficient taxonomic importance to be considered a separate genus.

As early as 1914 Raineri (6) reported the production by Strampelli in Italy of hybrids between *Triticum villosum* (*Haynaldia*) and *Triticum*. Tschermak (7, 8, 9) also obtained hybrids between *T. villosum* and several species of *Triticum*, one of which (*T. villosum* × *T. turgidum*) was fertile.

Bleier (2), Tschermak (8, 9), Tschermak-Seysenegg (10), and Oehler (5) reported hybrids between *Triticum villosum* and *Aegilops*. Only recently (1933) Verushkine and Shechurdine (11) reported the hybridization of *Triticum* with *Agropyron*.

The present paper records results from the hybridization of *Haynaldia villosa* (L.) Schur. with different species of *Triticum* and with *Secale fragile* L. Illustrations of the parents and hybrids, together with detailed morphological descriptions, illustrations, and data on fertility, are also presented.

¹ Received for publication July 11, 1935; issued January 1936.

² Reference is made by number (italic) to Literature Cited, p. 709.

MATERIAL AND METHODS

The seed of *Triticum dicoccoides* Koern., *T. dicoccum* Schrank, *T. durum* Desf., *T. turgidum* L., *T. polonicum* L., *T. vulgare* Vill.,¹ *T. compactum* Host, *T. spelta* L., and *Secale cereale* L. were obtained from the collection of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. The seed of *T. aegilopoides* Forsk., *T. timopheevi* Zhuk., *Secale fragile*, *S. cereale* subsp. *ancestrale* Zhuk., and *Haynaldia villosa* were obtained from Dr. P. M. Zhukovsky, of the Bureau of Applied Botany and Genetics, Union of Soviet Socialist Republics.

The crosses herein reported were made in the spring of 1930 in a greenhouse on the Arlington Experiment Farm, near Washington, D. C., and the parental and hybrid plants were likewise grown there. Preparatory to hybridization several upper and lower spikelets on a head were excised before blooming. Emasculation of the remaining flowers was then effected and the head enclosed in a glassine bag. Several days later when the stigmas had reached the stage of receptivity the glassine bags were removed and pollinations made, after which the glassine bags were again replaced until maturity. *Haynaldia villosa* was used as the pollen parent only in all of the crosses herein recorded.

To avoid unfair comparisons of characters subject to variation when plants are grown under different environments, all of the parents and hybrids were grown together in the same greenhouse in 6-inch unglazed earthen pots filled with a uniform mixture of composted soil during the same period of a single year.

Table 1 gives a list of the crosses and the results obtained.

TABLE 1.—Crosses made with *Haynaldia villosa* as the male parent, and results obtained

Female parent	Flowers pollinated	Seeds obtained	Seeds sown	Plants obtained
	Number	Number	Number	Number
<i>Triticum aegilopoides</i>	160	41	10	9
<i>T. timopheevi</i>	64	4	2	2
<i>T. dicoccoides</i> var. <i>spontaneonigrum</i>	116	20	17	7
<i>T. dicoccum</i> var. <i>Khapli</i>	188	14	5	1
<i>T. dicoccum</i> var. <i>Black Winter</i>	32	0	0	0
<i>T. durum</i> var. <i>Arnautka</i>	110	14	5	5
<i>T. durum</i> var. <i>Mindum</i>	96	8	3	1
<i>T. durum</i> var. <i>Kubanka</i>	82	15	7	3
<i>T. turgidum</i> var. <i>Alaska</i>	156	69	18	15
<i>T. polonicum</i> var. <i>C. I. 7498</i> ¹	140	13	5	2
<i>T. vulgare</i> var. <i>C. I. 6223</i> ¹	325	8	8	1
<i>T. vulgare</i> var. <i>Hard Federation</i>	90	0	0	0
<i>T. vulgare</i> var. <i>Velvet Chaff</i>	120	0	0	0
<i>T. vulgare</i> var. <i>Nittany</i>	400	1	1	0
<i>T. compactum</i> var. <i>Coppel</i>	130	2	2	0
<i>T. spelta</i> var. <i>Alstrom</i>	95	0	0	0
<i>Secale cereale</i> var. <i>Abruzzes</i>	146	0	0	0
<i>S. cereale</i> subsp. <i>ancestrale</i>	80	0	0	0
<i>S. fragile</i>	70	35	14	7

¹ Accession number of Division of Cereal Crops and Diseases.

² Died before formation of third leaf.

The more gross characters were studied by simple inspection, while the less gross, such as trichomes, required the use of a binocular dissecting microscope accurately to record form and dimensions. Because of the great variation in their length, diameter, and distribution the trichomes were studied in considerable detail. To differen-

³ According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L., but as *T. vulgare* is in general use among agronomists, cereal pathologists, and geneticists, the writer gives preference to the latter form.

tiate the variations of more or less similar morphological structures the terms used in this paper are defined as follows:

A *trichome* is any epidermal hair structure.

A *papilla* is a minute, usually slightly pointed beadlike protuberance 0.02 to 0.05 mm at its greatest diameter and 0.02 to 0.05 mm in perpendicular extension. The small papillae shown in plate 1, A, were found on the exterior surfaces of the glumes and lemmas of various species of *Triticum* and *Secale*. The large papillae shown in plate 1, B, are 0.025 to 0.05 mm in diameter and 0.02 to 0.05 mm in length and were found on the glumes and lemmas of *Haynaldia villosa*. Plate 2, D, shows large papillae found on the lemmas of *Haynaldia villosa*.

An *asperite* is a short, tapering, rigid antrorse point or sharp protruberance. The asperites shown in plate 1, C, are 0.03 to 0.2 mm in diameter and 0.06 to 0.5 mm in length and were found on the awns and on the exterior surfaces of the glumes, lemmas, and leaf edges of species of *Triticum*, *Secale*, and *Haynaldia*. Plants bearing small asperites or rigid antrorse points are designated scabrid.

A *spur asperite* is a large asperite which resembles in shape the horny appendage on the leg of a cock. The spur asperites shown in plate 1, D, are 0.07 to 0.3 mm in diameter and 0.5 to 1.6 mm in length and are found on the keels of lemmas of *Secale* species. Plants bearing spur asperites are designated scabrous.

A *superasperite* is a greatly enlarged asperite, 0.05 to 0.2 mm in diameter and 0.6 to 3 mm long. Superasperites are found on the glumes of *Triticum polonicum*, *T. timopheevi*, and the F₁ hybrids resulting from the hybridizing of *Haynaldia* with all of the species of *Secale* and *Triticum* except *T. polonicum*. Plate 1, E, shows superasperites produced on the glume and lemma keels of an F₁ hybrid resulting from the crossing of *T. turgidum*, which possesses asperites on its glume and lemma keels like those shown in plate 1, C, with *H. villosa*, which possesses bristles on its glume and lemma keels like those shown in plate 1, G. Plate 1, F, shows superasperites produced on the lemma keels of hybrids between *S. fragile*, which possesses asperites on its lemma keels like those shown in plate 1, D, and *H. villosa*, which possesses bristles on its lemma keels like those shown in plate 1, G.

A *bristle* is a stiffish, resilient, acicular process 0.02 to 0.05 mm in its greatest diameter and 0.2 to 5.0 mm in length. It is usually thicker in the middle than at either end. The bristles illustrated in plate 1, G, are 0.02 to 0.05 mm in diameter and 0.2 to 5.0 mm in length and are found on the lateral edges of the rachis internodes and usually in a tuft at the base of the spikelets of species of *Triticum*, *Secale*, and *Haynaldia*. They are also present in tufts on the keel of the glume and apex of the lemma of *Haynaldia*.

A *hair* is a slender flexible process 0.015 to 0.1 mm in diameter and 0.1 to 6.0 mm in length. From its greatest diameter at the base it tapers to a point. Hairs 0.25 to 5 mm in length and 0.03 to 0.09 mm in width are found on the auricles of species of *Triticum*, *Secale*, and *Haynaldia*. The hairs illustrated in plate 1, H, are 6 mm long and 0.07 to 0.09 mm in diameter at the base. These long hairs are found on the auricles of hybrids of *T. polonicum* and *T. timopheevi* with *H. villosa*. The hairs illustrated in plate 1, I, are 0.02 to 0.04 mm in diameter and 0.7 to 1.7 mm in length and are located on the lodicules, edges, and upper and lower surfaces of the leaf blades and the edges and exterior surfaces of the leaf sheaths of *Triticum*, *Haynaldia*, and *Secale*. The hairs shown in plate 1, J, are 0.015 to 0.03 mm in diameter and 0.1 to 0.2 mm long and are found on the leaves and nodes of some species of *Triticum*. This type of hairiness is referred to as puberulence. The hairs illustrated in plate 1, K, are 0.015 to 0.03 mm in diameter and 0.3 to 0.7 mm in length and may usually be observed on the exterior surfaces of the glumes, lemmas, and paleas of certain varieties of *Triticum* and *Secale*. This type of hairiness is referred to as pubescence.

THE PARENTS

HAYNALDIA VILLOSA

The plants of *Haynaldia villosa* have an upright habit of growth and in the writer's cultures have attained an average height at maturity of 124.5 cm. The culms are hollow at a point 2.5 cm below the head, glaucous, glabrous, and purple-pigmented (pl. 2, A; pl. 3, C). The nodes are glabrous.

The leaf blades are 7 mm wide and covered on the upper and lower surfaces with soft hairs 0.5 to 1 mm long. The edges of the leaf blades are scabrid and beset with hairs 1 to 2 mm long. The leaf sheaths are striate, usually weakly purple-pigmented, glaucous, and glabrous. One plant was observed with a trace of hairs on one leaf sheath. No hairs are present on the overlapping edges of the leaf sheaths. The auricles are 1.5 to 2 mm long and are beset with a few hairs 4 mm or less in length. The ligules project 3 mm from the point of attachment to the leaf. No hairs are present on the upper surface of the leaf blade immediately above the ligule (pl. 4, C).

The spikes are extremely fragile, 10 to 14 mm wide across the two-ranked face, 7 to 9 cm long, and composed of from twenty to thirty-two 2- to 3-flowered spikelets (fig. 1, C). The two lower florets of a spikelet usually produce kernels that average 4.7 mm in length (pl. 2, H). At maturity the rachis breaks with a clean fracture at its joints into small segments, at the apex of each of which is attached a spikelet (pl. 5, C).

The spikelets are 5 to 6 mm wide and 12 to 15 mm long exclusive of the awns (pl. 5, C). On the exterior side of the spikelet at the base are a few short bristles 0.5 mm or less in length (pl. 6, C). At the base of the spikelet on the side facing the rachis are also a few short bristles 0.3 mm or less in length (pl. 7, C). These bristles sometimes are grouped together, forming a small tuft.

The rachis internodes are obovate, 2 to 3 mm long, and each is glabrous except for a prominent tuft of bristles 1 to 3 mm long, which is located on the lower half of the two lateral edges (pl. 8, C).

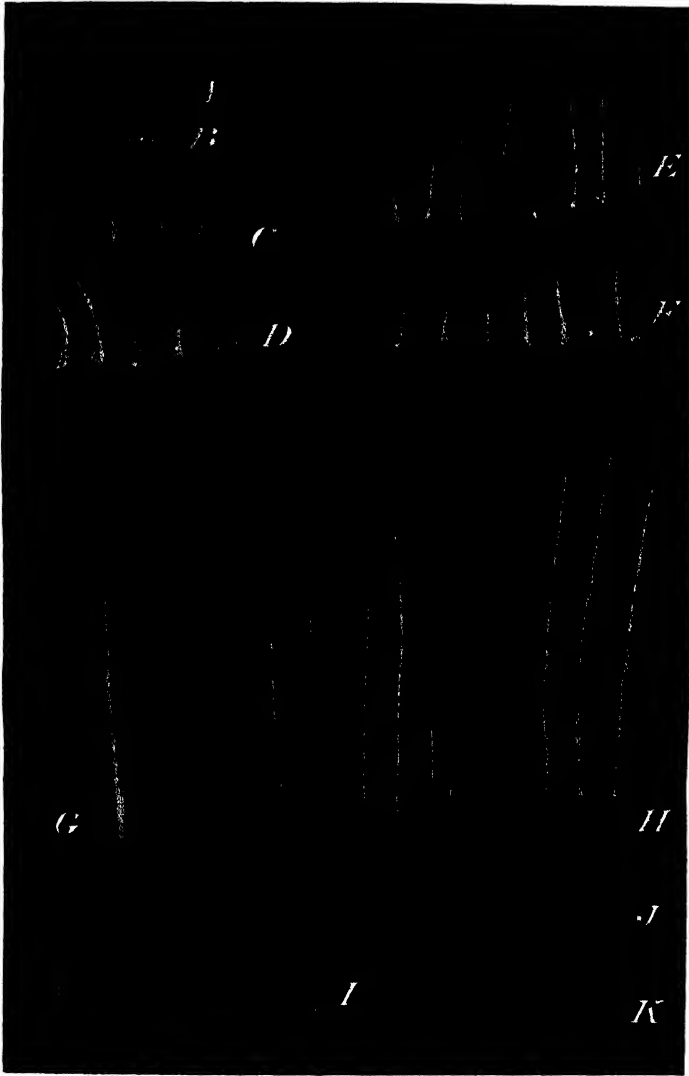
The glumes are glaucous, partly papillate, 1 to 2 mm wide, and 5 to 6 mm long exclusive of the awn and possess four nerves which converge toward the apex of the glume, where they fuse and form a purple scabrid awn 1 to 3 cm long (pl. 5 C). The two central nerves form distinct keels that are separated by a longitudinal channel about 1 mm wide at the center (fig. 1, C; pl. 5, C). Each glume has two keels, which are beset with tufts of bristles 1 to 3 mm long (pl. 5, C). The bristles extend upward farther on the front keel and are more abundant than on the rear keel. Sometimes the keels of the glumes may be completely devoid of bristles and possess only asperities or papillae, or both of these may be present together. The glume shoulders are wide, elevated, rounded, and nondentate (pl. 5, C).

The lemmas are 2 mm wide, 11 to 13 mm long, and papillate on their convex surfaces. Each has five nerves, the middle one of which forms a keel that terminates in a purple scabrid awn 3 to 4.5 cm long (pl. 5, C; pl. 2, D). At the apex of the lemma to the right and left of the awn is a short tooth 1 to 2 mm long (pl. 5, C). Some of the nerves of the lemma are scabrid. At the apex of the lemma on the keel are several tufts of bristles (pl. 5, C).

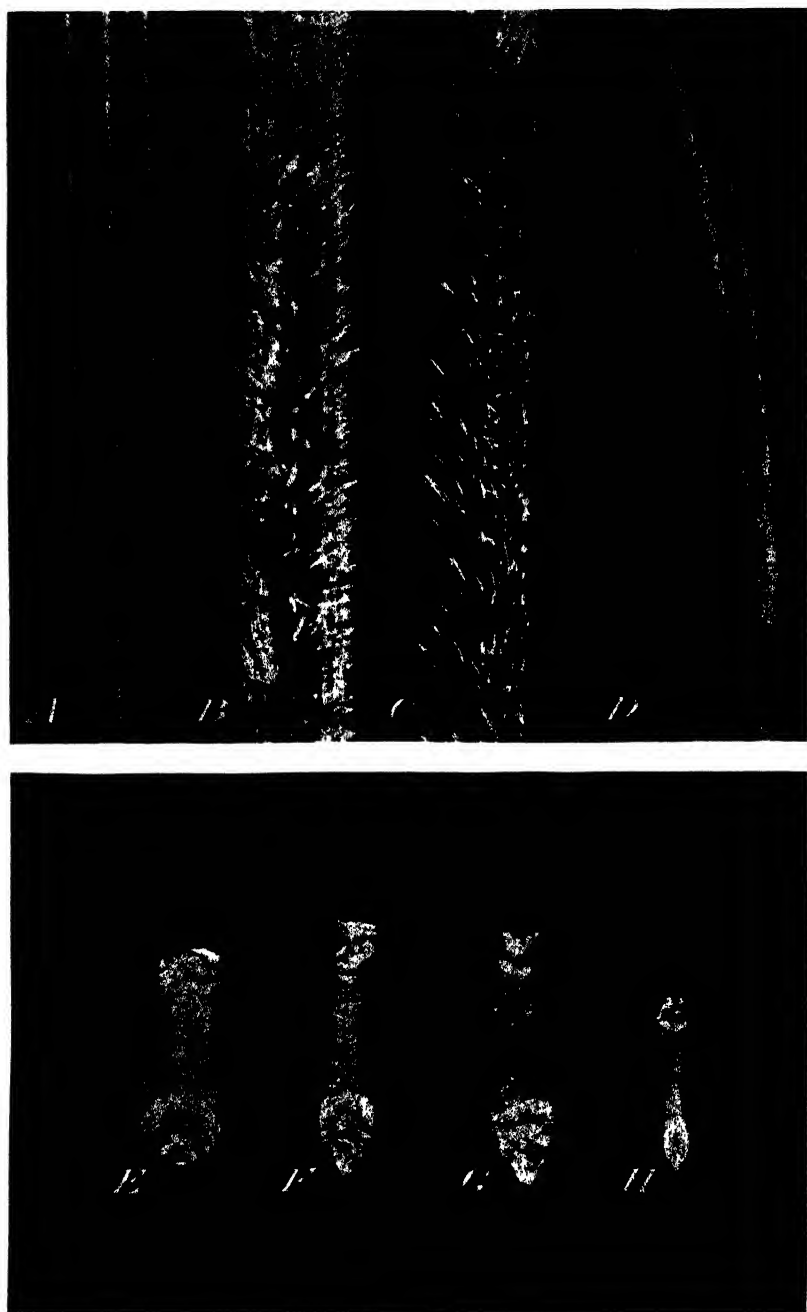
The paleas are entire, not split. The lodicules are 0.9 mm wide and 2 mm long and are beset with hairs 0.05 mm long.

TRITICUM AEGILOPOIDES

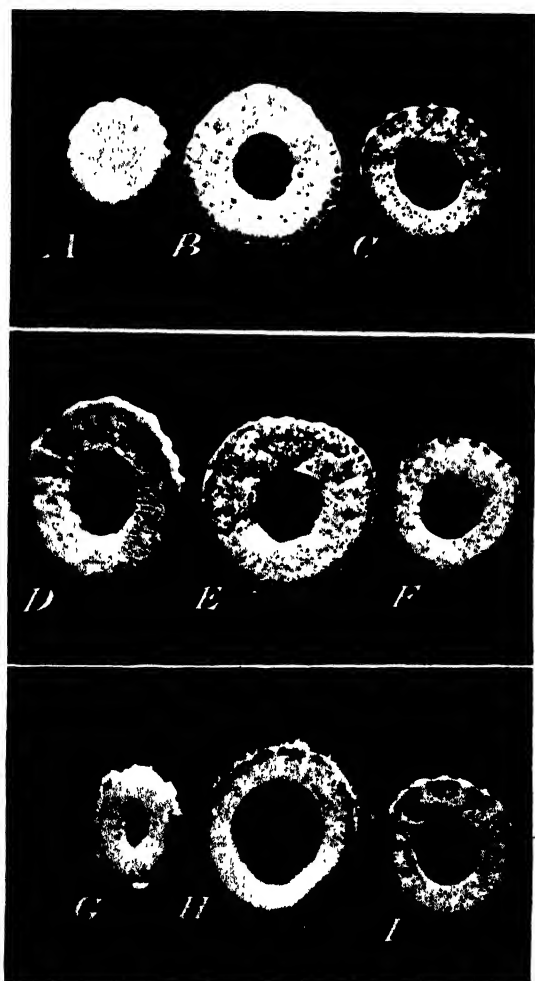
The plants of *Triticum aegilopoides* have an upright habit of growth and attain an average height at maturity of 93 cm. The culms are nonglaucous, without purple pigment, papillate, and solid at a point 2.5 cm below the head (pl. 3, A). The nodes are abundantly pubescent with hairs 1 mm or less in length.



Trichomes: A, Papillae on glumes and lemmas of *Triticum* and *Secale*; B, papillae on glumes and lemmas of *Haynaldia villosa*; C, asperites on glumes and lemmas of *Triticum*, *Secale*, and *Haynaldia*; D, spur asperites on lemma keels of *Secale*; E, superasperites on glume and lemma keels of F₁ hybrids of *T. iurgidum* var. Alaska \times *H. villosa*; F, superasperites on lemma keels of F₁ hybrids of *S. fragile* \times *H. villosa*; G, bristles on glumes and lemma keels of *H. villosa* and on spikelets at the base and edges of rachis internodes of *Triticum*, *Secale*, and *Haynaldia* spp.; H, hairs on auricles of *H. villosa*, *S. fragile*, and all of the species of *Triticum* used except *T. polonicum*; I, hairs on lodicules, leaves, and leaf sheaths of certain species of *Triticum*, *Secale*, and *Haynaldia*; J, hairs on leaf blades and nodes of certain species of *Triticum*; K, hairs on glumes, lemmas, and paleas of certain varieties of *Triticum* and *Secale*. $\times 10$.



Peduncles: A, *Haynaldia villosa*, B, F₁ of *Secale fragile* × *H. villosa*, C, *S. fragile*, D, *Haynaldia villosa* × about 15 Kernels E, *Triticum turgidum* var Alaska, F, F₁ of *T. turgidum* × *H. villosa*; G, F₂ of *T. turgidum* × *H. villosa*; H, *H. villosa* × about 4



Cross sections of culms at a point 2.5 cm below spike. A, *Triticum aegilopoides*; B, F_1 of *T. aegilopoides* \times *Haynaldia villosa*; C, *H. villosa*; D, *T. durum* var. *Kubanka*; E, F_1 of *T. durum* \times *H. villosa*; F, *H. villosa*; G, *Secale fragile*; H, F_1 of *S. fragile* \times *H. villosa*; I, *H. villosa*. \times about 15.



Portions of leaf blades and sheaths with and without tufts of hairs behind ligules: *A*, *Secale fragile*; *B*, F_1 of *S. fragile* \times *Haynaldia villosa*; *C*, *H. villosa*. \times about 15.

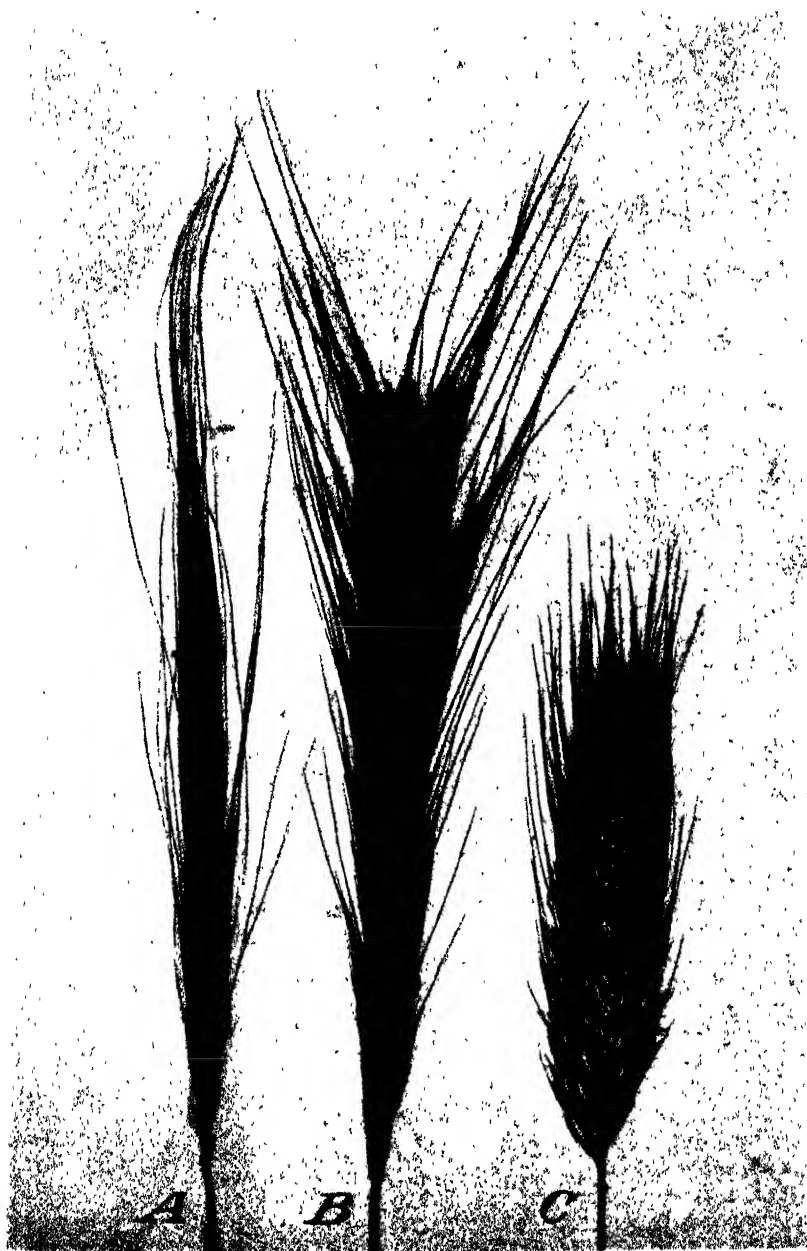


FIGURE 1.—Spikes: A, *Triticum aestivum*; B, F_1 of *T. aestivum* \times *Haynaldia villosa*; C, *H. villosa*. Natural size.

The leaf blades are 5 mm wide and covered on the upper and lower surfaces with soft hairs 0.5 to 1 mm long. The margins of the leaves are scabrid and beset with hairs 1 to 1.5 mm long. The leaf sheaths are pubescent, nonglaucous, without purple pigment, and the overlapping edges are beset with many hairs 1 to 1.5 mm long. The hairs on the overlapping edge of the upper leaf sheaths are few or entirely absent. The auricles are 1 mm long with hairs 3 to 5 mm in length. The ligules project 1 mm from the point of attachment to the leaf.

The spikes are extremely fragile, laterally compressed, 5 mm wide across the two-ranked face, and 7 to 9 cm long exclusive of awns, and consist of from twenty to forty 2-flowered spikelets (fig. 1, A). Each spikelet produces a very small laterally compressed kernel. At maturity the rachis breaks with a clean fracture at its joints into small segments, at the apex of each of which is attached a spikelet (pl. 5, A).

The spikelets are 2.5 to 2.8 mm wide and 9 mm long, exclusive of awns (pl. 5, A). On the exterior side of the spikelet at its base is a prominent tuft of bristles which are 0.5 to 3.0 mm long (pl. 5, A). At the base of the spikelet on the side facing the rachis is a small tuft of hairs which are 0.5 mm or less in length.

The rachis internodes are oblong, 2.7 mm long, and glabrous except on the two lateral edges, which are fringed with numerous bristles 0.5 to 3.0 mm long (pl. 5, A).

The glumes are nonglaucous, 1 mm wide, 5 to 7 mm long exclusive of beaks, papillate on their convex surfaces, and possess 4 or 5 nerves, 2 of which are prominent and beset with asperities 0.15 mm in length. The rear one of the 2 prominent nerves forms a keel that terminates in a short beak 1.5 mm long (pl. 5, A). At the apex of the front nerve is a short, pointed tooth 0.5 mm long (pl. 5, A). The glume shoulders are narrow and oblique, with a sharp-pointed tooth (pl. 5, A).

The lemmas are 1.5 to 1.8 mm wide, 7 to 9 mm long exclusive of the awns, 8- to 10-nerved, partly keeled, and abundantly papillate on their convex surfaces. At the apex of each are two prominent scabrid points or teeth 1 mm long, between which is a long, yellow, scabrid awn 2.0 to 9.0 cm long (pl. 5, A).

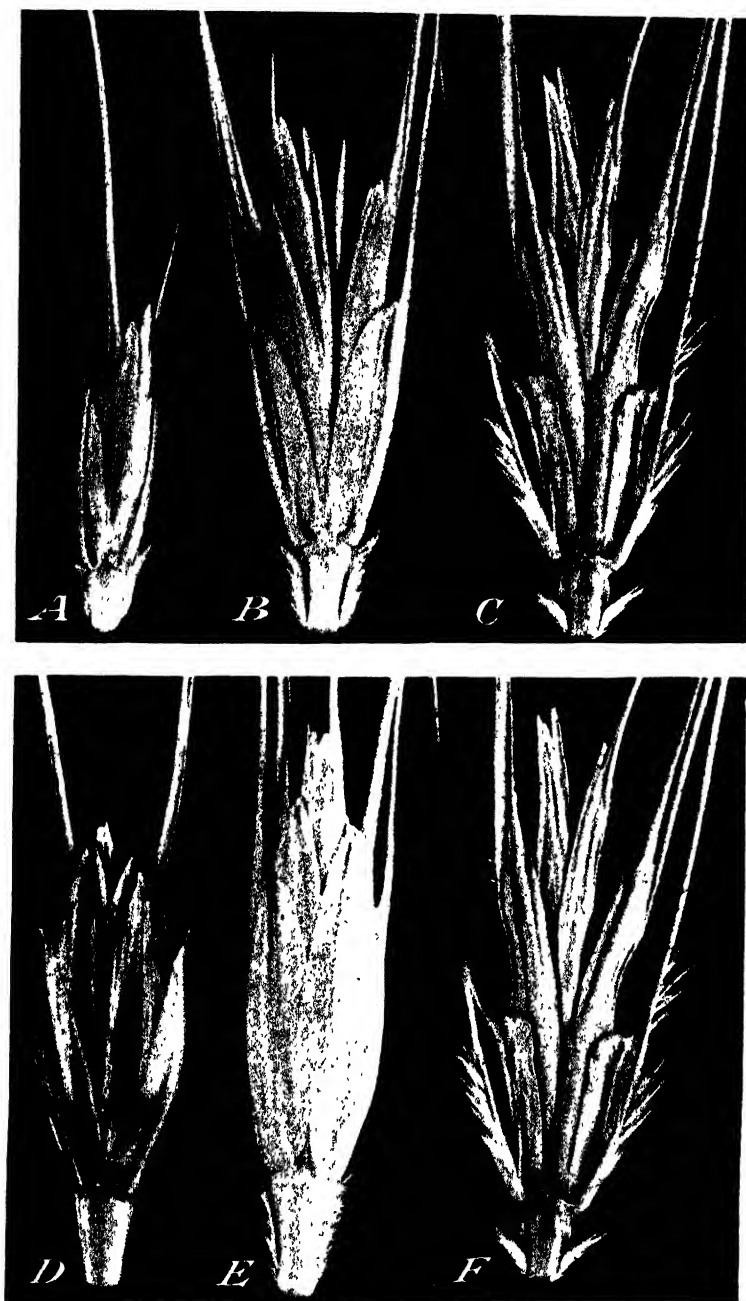
The paleas of the fertile florets are split longitudinally through the middle from the apex to the base.

The lodicules are 0.33 mm wide and 0.8 mm long and are beset with hairs 0.33 mm long.

TRITICUM TIMOPHEEVI

The plants of *Triticum timopheevi* have an upright habit of growth and attain an average height at maturity of 142.2 cm. The culms are glaucous, devoid of purple pigment, and papillate for a distance of several centimeters below the head. The culms are hollow at a point 2.5 cm below the head. The nodes are puberulent, the hairs being 1 mm or less in length. The puberulence may extend 3 mm below the node.

The leaf blades are 8 mm wide, slightly scabrid, and covered on the upper and lower surfaces with hairs 2.5 mm or less in length. The edges of the blades are scabrid and possess hairs 2 mm or less in



Spikelets: A, *Triticum aegilopoides*; B, *T. aegilopoides* \times *Haynaldia villosa*; C, *H. villosa*; D, *T. dicoccum* var *Khapli*; E, F₁ of *T. dicoccum* \times *H. villosa*; F, *H. villosa*. \times about 4.



Basal bristles on exterior side of spikelet. 1, *Triticum dicoccoides* var *spontaneonigrum*, B, F₁ of *T. dicoccoides* × *Haynaldia villosa*, C, *H villosa*. × about 15.



Basal bristles on side of spikelet facing rachis. A, *Triticum dicoccoides* var. *spontaneonigrum*. B, F₁ of *T. dicoccoides* × *Haynaldia villosa*. C, H₁ *villosa*. × about 15.



Rachis internodes: A, *Triticum dicoccoides* var *spontaneonigrum*. B, F, of *T. dicoccoides* \times *Haynaldia villosa*, C, *H. villosa* \times about $\frac{1}{15}$

length. The leaf sheaths are without anthocyanin pigment, glaucous, and usually scabrid. The upper leaf sheaths usually are devoid of hairs, while the lower ones may possess them in abundance 2 mm or less in length. The overlapping edge of each leaf sheath is scabrid and beset with hairs. The auricles are 1 mm long and beset with several hairs 5 mm or less in length. The ligules project 1 mm from the point of attachment to the leaf.

The spikes are 11 to 13 mm wide across the two-ranked face, 6 to 8 cm long exclusive of the awns, and composed of from eighteen to twenty-one 2- or 3-flowered spikelets (fig. 2, A). At maturity, the head may break at the base as a unit just above the uppermost sterile spikelet (pl. 9, D). However, with slight pressure the rachis breaks with a slightly ragged fracture into small segments, at the apex of each of which is attached a spikelet.

The spikelets are 7 to 8 mm wide and 16 to 18 mm long exclusive of the awns or beaks (pl. 9, D). Each spikelet usually contains two slender kernels. On the exterior side of the spikelet at the base is a prominent tuft of bristles, which are 2 mm or less in length and extend to the fringed edges of the rachis internodes to which the spikelet is attached (pl. 9, D). At the base of the spikelet on the side facing the rachis is a smaller tuft of bristles which are 1 mm or less in length.

The rachis internodes are 2 mm long, partly puberulent, and papillate on the convex and concave surfaces, and the edges are fringed with numerous bristles 1 mm or less in length (pl. 9, D).

The glumes are 2 to 3 mm wide, 9 mm long exclusive of the awns, densely pubescent, and glaucous, and each possesses 5 to 7 nerves, the most prominent one of which forms the keel, which is beset on its upper edges with asperites 0.5 mm or less in length (pl. 9, D). Some of the hairs on certain portions of the glume, especially on the keel and other prominent nerves, are thicker and longer than the others. These thick hairs resemble superasperites and attain a maximum length of 1 mm. The keel is very sharp and is terminated by a beak 5 mm or less in length. The glume shoulders are narrow, with a pointed tooth (pl. 9, D).

The lemmas are 3 to 3.5 mm wide, 13 to 14 mm long exclusive of the awns, pubescent on exposed portions, and papillate on portions covered by the glumes. They possess 8 to 9 inconspicuous nerves, the central one of which is partly keeled and terminates in a brown to black scabrid awn 11 cm or less in length. The hairs on the lemma at the apex may vary in length and thickness. These long hairs resemble superasperites and attain a maximum length of 1 mm.

The paleas are entire, not split. The lodicules are 0.6 mm wide and 1.8 mm long and are beset with hairs 0.65 mm long.

TRITICUM DICOCOIDES

The plants of *Triticum dicoccoides* var. *spontanconigrum* have an upright habit of growth and attain an average height at maturity of 76.2 cm. The culms are not glaucous, solid at a point 2.5 cm. below the head, and without purple pigment. The nodes are abundantly covered with deflexed hairs 0.2 to 0.4 mm long.

The leaf blades are 6 to 8 mm wide and puberulent on the upper and lower surfaces. The edges of the leaf blades are slightly



FIGURE 2.—Spikes: A, *Triticum timopheevi*; B, F₁ of *T. timopheevi* × *Haynaldia villosa*; C, *H. villosa*. Natural size.

scabrid and devoid of hairs. The leaf sheaths are nonglaucous, without purple pigment, and glabrous except on the edges, which are fringed with hairs. The auricles are 1 mm long and are beset with hairs 2 to 3 mm long. The ligules project about 3 mm from the point of attachment to the leaf.

The spikes are extremely fragile, laterally compressed, 8 to 9 mm wide across the two-ranked face, and 7 to 9 cm long exclusive of the awns, and consist of from sixteen to eighteen 2- or 3-flowered spikelets, each of which normally contains two kernels (fig. 3, *A*). At maturity the rachis disarticulates at its joints with a clean fracture into small segments at the apex of each of which is attached a spikelet (pl. 10, *A*).

The spikelets are 4 to 5 mm wide and 15 to 17 mm long exclusive of the awns. On the exterior side of the spikelet at the base is a conspicuous tuft of bristles 3 to 5 mm long (pl. 6, *A*). At the base of the spikelet on the side facing the rachis is a small tuft of bristles 2 mm or less in length (pl. 7, *A*).

The rachis internodes are nonglaucous, oblong, 4 mm long, and glabrous except on the two lateral edges, which are fringed along their entire length with numerous bristles 0.5 to 5 mm long (pl. 8, *A*).

The glumes are nonglaucous, 2 mm wide, 12 to 13 mm long exclusive of the beak, and white with black streaks which sometimes appear as a solid mass of black pigment (pl. 10, *A*). They possess two prominent nerves, the rear one of which is beset with large asperities, is papillate, and forms a keel that terminates in a short beak 2 mm long, while at the apex of the forward nerve is a very short tooth less than 0.25 mm long (pl. 10, *A*). One or two less conspicuous nerves may be present also on the glumes. The glume shoulders are narrow and oblique, with a very short, pointed tooth (pl. 10, *A*).

The lemmas are 2 mm wide, 14 to 15 mm long exclusive of the awn, partly papillate, and slightly scabrid on their exposed surfaces. Each terminates in three points, the center one of which is extended into a scabrid, black awn, 10 to 17.5 cm long (pl. 10, *A*).

The paleas are entire, not split. The lodicules are 0.9 mm wide and 1.6 mm long and are beset with hairs 0.6 mm long.

TRITICUM DICOCCUM

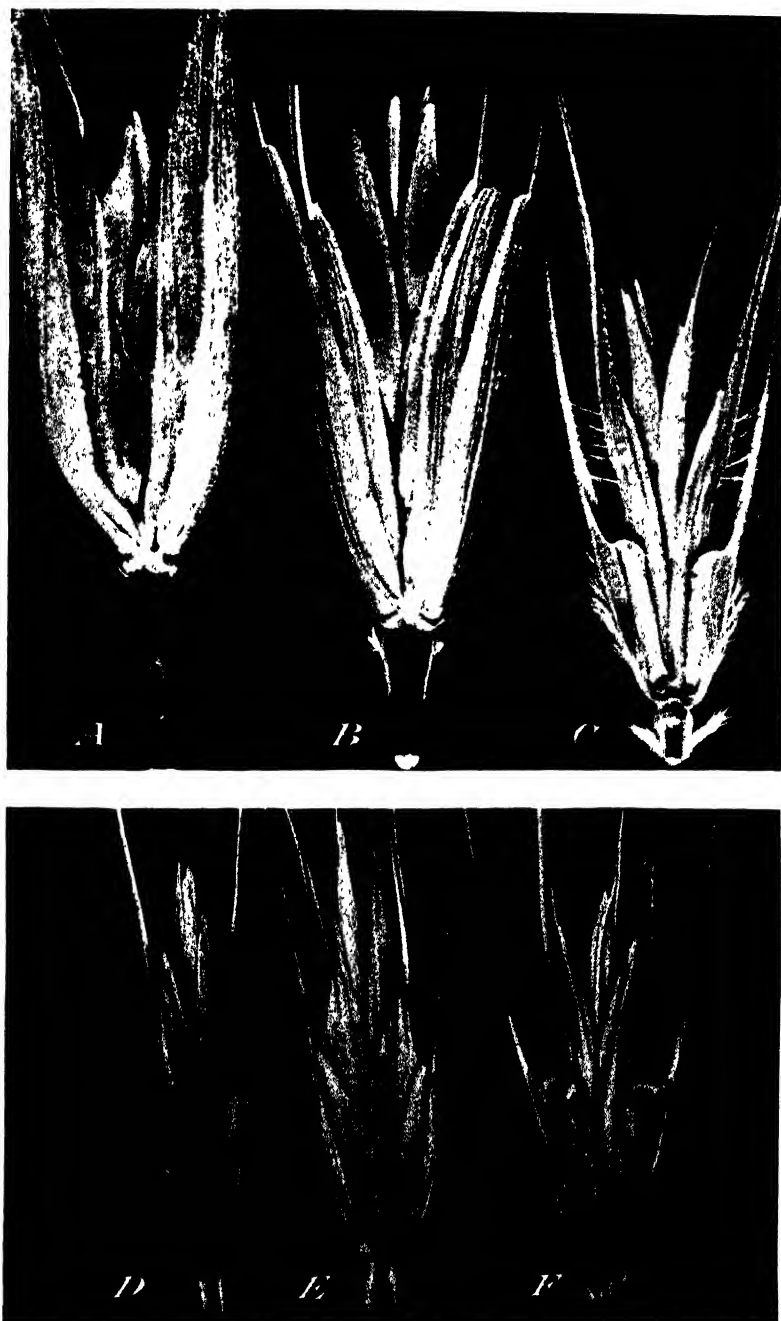
The plants of *Triticum dicoccum* var. Khapli have an upright habit of growth and attain an average height at maturity of 68.6 cm. The culms are glabrous, nonglaucous, without purple pigment, and solid at a point 2.5 cm below the head. The nodes are puberulent.

The leaf blades are 10 to 12 mm wide and puberulent on both the upper and lower surfaces. The margins of the leaf blades are scabrid and devoid of hairs. The leaf sheaths are slightly glaucous, without purple pigment, and glabrous on the exterior surfaces, while the lateral edges of the sheaths are devoid of hairs. The auricles are 1.5 to 2.0 mm long with a few hairs 0.7 mm or less in length. The ligules project 2.0 mm from the point of attachment to the leaves.

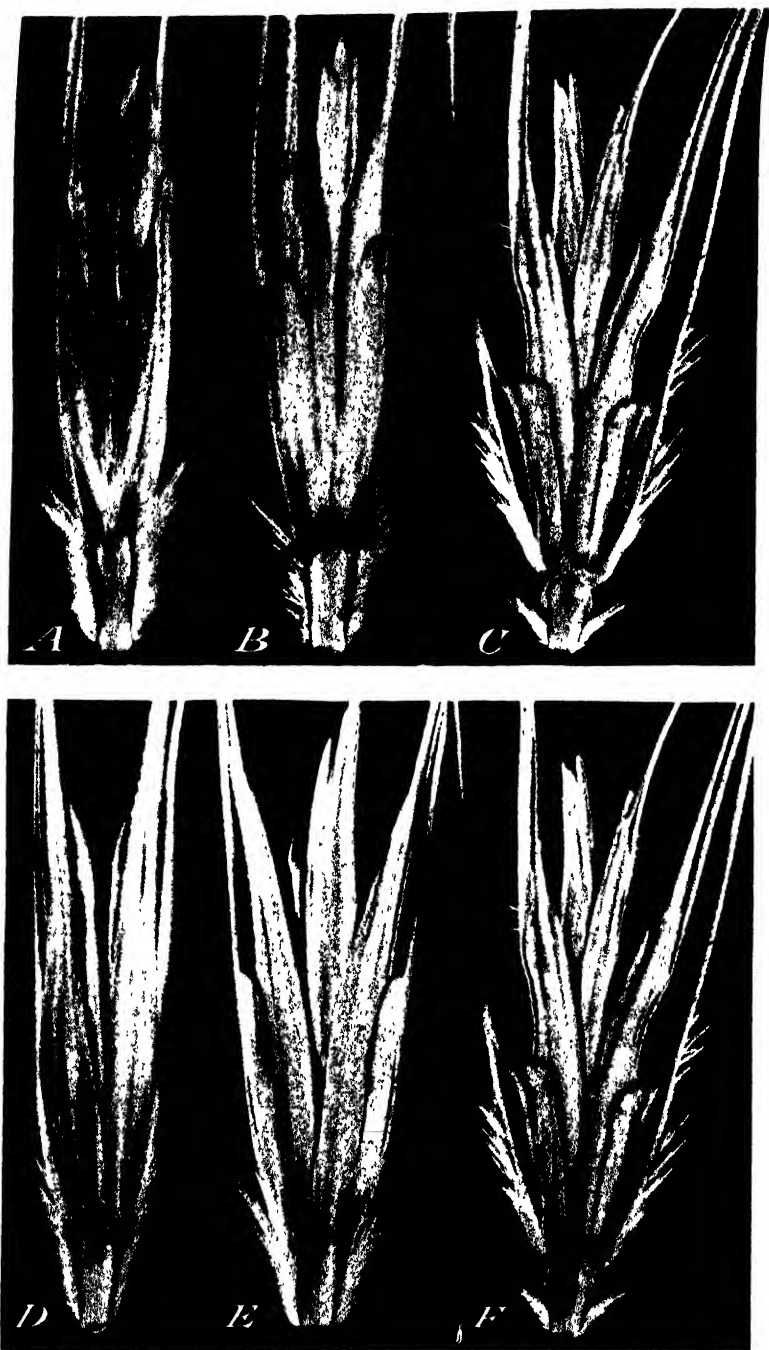
The spikes are fragile, laterally compressed, 8 to 10 mm wide across the two-ranked face, 7 to 9 cm long, and consist of from sixteen to twenty-four 2- or 3-flowered spikelets, each of which normally produces two kernels (fig. 4, *A*). At maturity the rachis usually breaks with an imperfect or ragged fracture at its joints into small segments, at the apex of each of which is attached a spikelet (pl. 5, *D*).



FIGURE 3.—Spikes: A, *Triticum dicoccoides* var. *spontanconigrum*; B, F_1 of *T. dicoccoides* \times *Haynaldia villosa*; C, *H. villosa*. Natural size.



Spikelets. A, *Triticum polonicum* (C. I. 7498); B, F₁ of *T. polonicum* × *Haynaldia villosa*; C, *H. villosa*; D, *T. timopheevi*; E, *T. timopheevi* × *H. villosa*; F, *H. villosa*. × about 3.



Spikelets: A, *Triticum dicoccoides* var. *spontaneonigrum*; B, F₁ of *T. dicoccoides* × *Haynaldia villosa*; C, *H. villosa*; D, *Secale fragile*; E, F₁ of *S. fragile* × *H. villosa*; F, *H. villosa* × about 4.



Spikelets. A, *Triticum turgidum* var. Alaska; B, F₁ of *T. turgidum* × *Haynaldia villosa*; C, *H. villosa*; D, *T. durum* var. Kubanka; E, F₁ of *T. durum* × *H. villosa*; F, *H. villosa*. × about 3.



Glumes: A, *Triticum turgidum* var. Alaska; B, F₁ of *T. turgidum* × *Haynaldia villosa*; C, *H. villosa*.
 × about 12. Lemmas: D, *Triticum turgidum* var. Alaska; E, F₁ of *T. turgidum* × *Haynaldia villosa*;
 F, *H. villosa*. × about 12.

The spikelets are 6 to 7 mm wide and 10 to 11 mm long exclusive of the awns (pl. 5, *D*). On the exterior side of the spikelet at the base is a prominent tuft of bristles which are 1 to 1.25 mm long (pl. 5, *D*). At the base of the spikelet on the side facing the rachis is also a small tuft of bristles which are 0.25 to 0.8 mm long.

The rachis internodes are weakly glaucous, 3 mm long, and glabrous except on the two lateral edges, which are fringed with numerous bristles 0.25 to 1 mm long (pl. 5, *D*).

The glumes are weakly glaucous, 2.5 mm wide, 9 to 10 mm long exclusive of the beak, papillate on their convex surfaces, and possess 6 to 7 nerves, two of which appear prominent. Of the 2 to 3 prominent nerves on each glume, the one nearest to the rachis forms a sharp keel which terminates in a short beak 0.5 mm long (pl. 5, *D*). The upper half of the keels is beset with short asperities, the lower portion being papillate. Occasionally the upper portion of the other prominent nerves are slightly scabrid. The glume shoulders are narrow and oblique, with a short, blunt tooth.

The lemmas are 3 mm wide and 10 to 11 mm long, 10 to 11 nerved, and papillate on their convex surfaces. At the apex of each lemma are two teeth or points less than 0.5 mm long between which is a yellow scabrid awn 3 to 9 cm long (pl. 5, *D*).

The paleas are entire, not split. The lodicules are 0.9 mm wide and 1.7 mm long and are beset with hairs 0.6 mm long.

TRITICUM DURUM

The plants of *Triticum durum* var. Kubanka have an upright habit of growth and attain an average height at maturity of 132 cm. The culms are glabrous, glaucous, without purple pigment, and hollow at a point 2.5 cm below the head (pl. 3, *D*). The nodes are glabrous.

The leaf blades are 8 to 10 mm wide and glabrous on the upper and lower surfaces. The edges of the leaf blades are scabrid and devoid of hairs. The leaf sheaths are glaucous, without purple pigment, glabrous on the exterior surfaces, and devoid of hairs on the lateral edges. The auricles are 1 to 2 mm long and devoid of hairs. The ligules project 2 to 2.5 mm from the point of attachment to the leaf.

The spikes are nonfragile, 7 to 8 mm wide across the two-ranked face, 7 to 8 cm long, and composed of from eighteen to twenty-four 2- to 4-flowered spikelets, each of which usually contains two kernels (fig. 5, *A*).

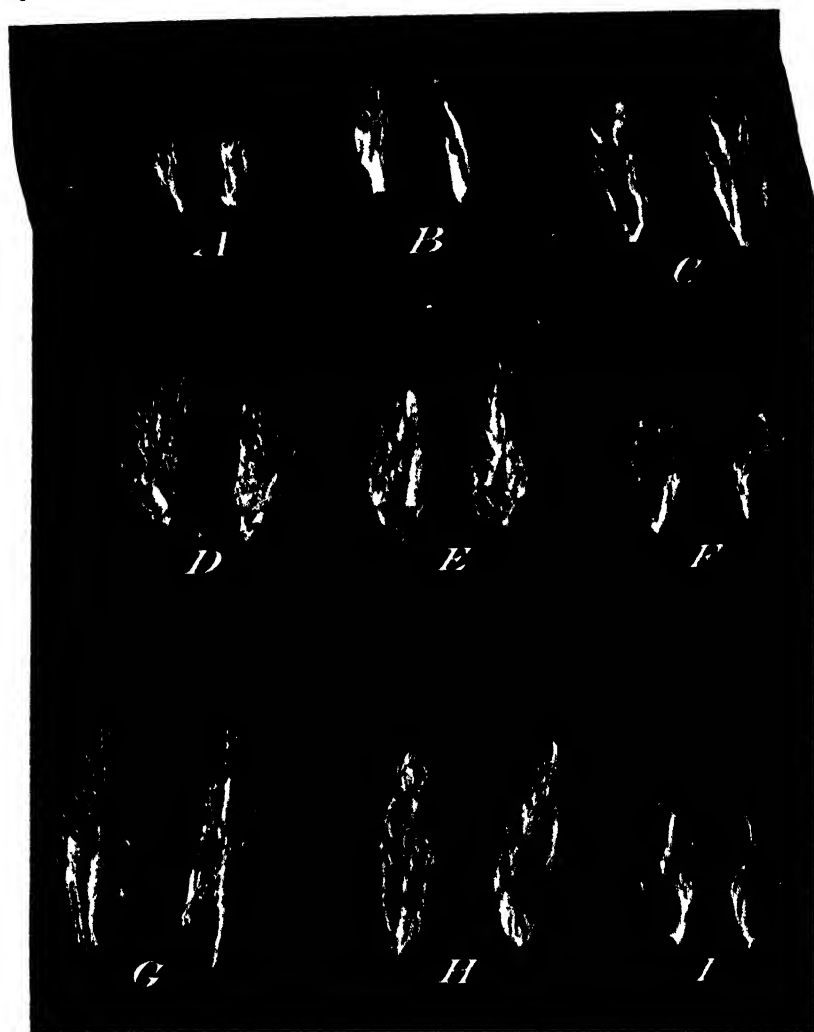
The spikelets are 10 to 12 mm wide and 12 to 14 mm long exclusive of the awns. On the exterior side of the spikelet at the base is a tuft of bristles 2 mm or less in length. At the base of the spikelet on the side facing the rachis is a tuft of bristles 0.3 mm or less in length.

The rachis is continuous. The rachis internodes are 3 to 5 mm long, partly glaucous, and glabrous except on the two lateral edges, which are fringed with short bristles 0.2 to 0.3 mm long near the base and 1 to 1.5 mm at the apex.

The glumes are 1.5 to 2 mm wide and 7 to 8 mm long exclusive of beak, papillate, glaucous on their convex surfaces, and possess 7 to 8 nerves, the most prominent of which forms a scabrid keel which



FIGURE 4.—Spikes: A, *Triticum dicoccum* var. Khapli; B, F₁ of *T. dicoccum* × *Haynaldia villosa*; C, *H. villosa*. Natural size.



Lodicules. *A*, *Triticum aegilopoides*; *B*, F_1 of *T. aegilopoides* \times *Haynaldia villosa*; *C*, *H. villosa*; *D*, *T. polonicum* (C 1. 7498); *E*, F_1 of *T. polonicum* \times *H. villosa*; *F*, *H. villosa*; *G*, *Secale fragile*; *H*, F_1 of *S. fragile* \times *H. villosa*; *I*, *H. villosa*. $\times 12\frac{1}{2}$



Glumes: A, *Secale fragile*; B, F₁ of *S. fragile* × *Haynaldia villosa*; C, *H. villosa*. Lemmas: D, *S. fragile*; E, F₁ of *S. fragile* × *H. villosa*; F, *H. villosa*. × about 13



FIGURE 5.—Spikes: A, *Triticum durum* var. Kubanka; B, F_1 *T. durum* \times *Haynaldia villosa*; C, *H. villosa*. Natural size.

terminates in a beak 1 to 2 mm long. The glume shoulders are narrow, slightly elevated, and nondentate (pl. 11, *D*).

The lemmas are 2 to 2.5 mm wide, 8 to 9 mm long exclusive of awns, papillate, glaucous on their upper exposed surfaces, and possess 1 to 13 nerves, the most prominent of which terminates in a yellow scabrid awn 10 to 16 cm long.

The lodicules are 0.9 mm wide and 1.3 mm long and are beset with hairs 0.5 mm long.

TRITICUM POLONICUM

The plants of *Triticum polonicum* (C. I. no. 7498) have an upright habit of growth and attain an average height at maturity of 124.5 cm. The culms are glabrous and hollow, with a small cavity at a point 2.5 cm below the head, glaucous, and without purple pigment. The nodes are puberulent and glaucous.

The leaf blades are 18 to 22 mm wide and practically devoid of hairs on the upper and lower surfaces. The margins of the leaf blades are scabrid and beset with short hairs less than 1 mm in length for a distance of 5 mm above the auricle. The leaf sheaths are glaucous, without purple pigment, glabrous on the exterior surfaces, and usually devoid of hairs. The auricles are 1 mm long with a few hairs 0.4 mm or less in length. The ligules project 1.5 to 2.0 mm from the point of attachment to the leaf.

The spikes are nonfragile, 12 to 14 mm wide across the two-ranked face, 11 to 13 cm long exclusive of the awns, and consist of from eighteen to twenty-two 3- or 4-flowered spikelets, each of which usually contains three long kernels (fig. 6, *A*).

The spikelets are 10 to 12 mm wide and 20 to 25 mm long exclusive of awns (pl. 9, *A*). On the exterior side of the spikelet at the base is a tuft of bristles 0.5 to 2.5 mm long (pl. 9, *A*). At the base of the spikelet on the side facing the rachis is a tuft of bristles 0.3 mm or less in length.

The rachis is continuous. The rachis internodes are 7 to 9 mm long, glaucous, and glabrous except on the two lateral edges, which are partly fringed with bristles 0.5 to 2.0 mm long (pl. 9, *A*).

The glumes are glaucous, 3 mm wide and 22 mm long, and possess 8 to 10 nerves, the most prominent of which forms a keel that terminates in a tooth or beak 1.5 to 5 mm long (pl. 9, *A*). A weaker secondary keel may also be developed. The keels and some of the nerves showing prominence at the apex of the glume are beset with long, slender asperites 0.5 mm or less in length. The convex surface of each glume on the membranous portion of the two wings and on the edge is pubescent (pl. 9, *A*). Other parts of the glume may be papillate or puberulent. The glume shoulders are narrow, elevated, and nondentate (pl. 9, *A*).

The lemmas are 4 mm wide, 20 to 28 mm long, glaucous, papillate, and pubescent on the exposed surfaces (pl. 9, *A*). Each lemma possesses 9 to 12 nerves, the most prominent of which terminates in a partially black, scabrid awn 9 to 12 cm long (fig. 6, *A*).

The paleas are entire, not split. The lodicules are 1 mm wide and 1.6 mm long and are beset with hairs 1.35 mm long.



FIGURE 6.—Spikes: A, *Triticum polonicum* (C. I. 7498); B, F_1 of *T. polonicum* \times *Haynaldia villosa*; C, *H. villosa*. Natural size.

TRITICUM TURGIDUM

The plants of *Triticum turgidum* var. Alaska have an upright habit of growth and attain an average height at maturity of 127.0



FIGURE 7.—Spikes: A, *Triticum turgidum* var. Alaska; B, F_1 of *T. turgidum* \times *Haynaldia villosa*; C, *H. villosa*. Natural size.

cm. The culms are glabrous, glaucous, without purple pigment, and solid at a point 2.5 cm below the head. The nodes are glabrous.

The leaf blades are 15 to 18 mm wide and abundantly puberulent on the upper surface, while the lower surface is only sparsely puberulent. The edges of the blades are nonscabrid and free from hairs.

The leaf sheaths are glaucous, without purple pigment, and glabrous on the surface and overlapping lateral edges. The auricles are 2 to 3 mm long and beset with a few short hairs less than 0.25 mm long. The ligules project 3 mm from the point of attachment to the leaf.

The spikes may be branched or simple, nonfragile, 8 to 12 cm long exclusive of the awns, and may vary in width from 12 to 35 mm across the two-ranked face (fig. 7, *A*).

The spikelets are 7 to 8 mm wide, 9 to 10 mm long exclusive of the awns, and generally contain two short plump kernels with a high dorsal arch or hump (pl. 11, *A*; pl. 2, *E'*). On the exterior side of the spikelet at the base is a tuft of bristles which are 3.0 mm or less in length and sometimes extend to the edges of the rachis internode to which the spikelet is attached (pl. 11, *A*). At the base of the spikelet on the side facing the rachis is a tuft of short bristles 0.5 mm or less in length.

The rachis is continuous. The rachis internodes are oblong, 2.5 to 3.0 mm long, glaucous, and glabrous, and the edges are fringed with numerous bristles 3 mm or less in length (pl. 11, *A*).

The glumes are 2.5 mm wide, 5 to 6 mm long exclusive of the beaks, papillate, glaucous on their convex surfaces, and possess four prominent nerves, the most conspicuous of which forms the keel and terminates in a short beak 0.5 mm or less in length. The keels throughout the entire length of the glumes are beset with asperites 0.2 mm or less in length (pl. 12, *A*). The exterior surface of the membranous portion of the two wings of the glume is partly puberulent. The glume shoulders are narrow, oblique, and nondentate (pl. 11, *A*).

The lemmas are 3 mm wide, 8 mm long exclusive of the awns, glaucous, slightly scabrid on their upper exposed surfaces, and possess 8 to 11 nerves. The most prominent nerve on each lemma terminates in a scabrid, black awn 6 to 11 cm long (pl. 12, *D*).

The paleas are entire, not split. The lodicules are 0.7 mm wide and 1.1 mm long and are beset with hairs 0.7 mm long.

SECALE FRAGILE

The plants of *Secale fragile* have an upright habit of growth and attain an average height at maturity of 76 cm. The culms are hollow, purple-pigmented, glaucous, and covered with hairs 1 mm or less in length for several centimeters immediately below the head (pl. 2 *C*; pl. 3, *G*). The nodes are puberulent.

The leaf blades are 6 to 7 mm wide and covered with hairs 1 to 1.5 mm long on their upper surfaces. The lower surfaces of the leaf blades are puberulent. The edges of the leaf blades are scabrid and beset with hairs 0.3 mm long for a distance of 5 mm above the auricle on the blade. Just above the ligule on the leaf blade is a prominent collar of hairs that do not exceed 2 mm in length. The upper leaf sheaths are glabrous, glaucous, and slightly purple-pigmented on the exterior surfaces. The lower leaf sheaths are hairy. The lateral edges of the sheaths are devoid of hairs. The auricles are 0.5 mm in length and possess very short hairs 0.4 mm or less in length. The ligules project 0.5 to 0.7 mm above the point of attachment to the leaf.

The spikes are extremely fragile, 7 to 8 mm wide across the two-ranked face, 8 to 11 cm long exclusive of the awns, and consist of

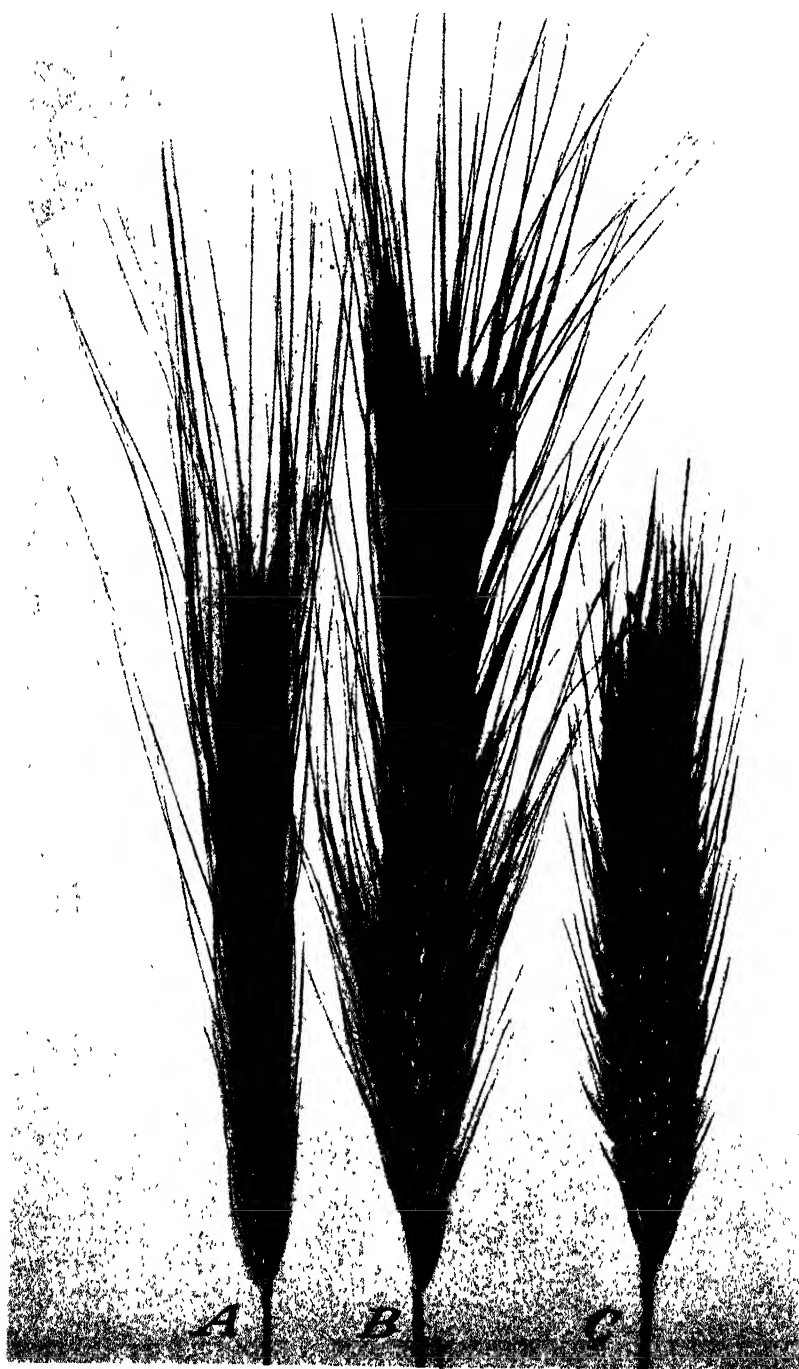


FIGURE 8.—Spikes: A, *Secale fragile*; B, F_1 of *S. fragile* \times *Haynaldia villosa*; C, *H. villosa*. Natural size.

twenty-six to thirty-six 2-flowered spikelets (fig. 8, A). Each spikelet usually produces two kernels. At maturity the rachis breaks with a clean fracture at its joints into small segments at the apex of each of which is attached a spikelet (pl. 10, D).

The spikelets are 5 mm wide and 14 to 16 mm long exclusive of the awns. On the exterior side of the spikelet at the base are a few extremely short bristles (pl. 10, D). At the base of the spikelet on the side facing the rachis are a short tuft and a collar of bristles which are 0.2 mm or less in length. The rachis internodes are 3 to 4 mm long, glaucous, and glabrous, and the two lateral edges are fringed with bristles 4 mm or less in length (pl. 10, D).

The glumes are slightly glaucous, subulate-pointed, 0.5 mm wide, 13 to 14 mm long exclusive of a scabrid awn 6 mm long, partly papillate, and scabrid on the exterior surfaces (pl. 10, D). Each glume has a keel which is densely beset with slender asperites 0.5 mm or less in length, which become longer and resemble hairs as they approach the base of the glume. The margins of the glumes are fringed with numerous slender hairs 1 mm or less in length. The glumes are sparsely beset with asperites. Glume shoulders are wanting (pl. 10, D).

The lemmas are 2.5 mm wide, 15 to 17 mm long exclusive of the awn, papillate, and 3- to 5-nerved; the most prominent nerve forms a keel beset throughout its entire length with somewhat uniformly separated and linearly arranged spur asperites 1 mm or less in length (pls. 1, D; 10, D). The keel is terminated by a partially purple-pigmented, scabrid awn 12 cm long. The edges of the lemma are beset with hairs 0.5 to 0.7 mm long. The exposed portion of the lemmas on and near the edges and at the apex is usually beset with asperites or spur asperites.

The paleas are entire, not split. The lodicules are 1.1 mm wide and 2.9 mm long and are beset with hairs 1.25 mm long.

THE F₁ HYBRIDS

TRITICUM AEGILOPOIDES × *HAYNALDIA VILLOSA*

The plants of the *Triticum aegilopoides* × *Haynaldia villosa* hybrid have an upright habit of growth and attain an average height at maturity of 116.8 cm. The culms are glaucous, nonpapillate, hollow at a point 2.5 cm below the head, and purple-pigmented (pl. 3, B). The nodes are sparsely puberulent.

The leaf blades are 6 to 7 mm wide and covered on the upper and lower surfaces with hairs 1 to 1.5 mm long. The edges of the leaf blades are scabrid and possess hairs 1.5 to 2 mm long. The leaf sheaths are glaucous, pubescent, slightly purple-pigmented on the exterior surface, and glaucous on the lateral edges. The auricles are 1.5 mm long with hairs 3 mm or less in length. The ligules project 1.5 mm from the point of attachment to the leaf.

The spikes are extremely fragile, 4 to 6 mm wide across the two-ranked face, 9 to 11 cm long exclusive of the awns, and composed of from thirty to forty-six 2- or 3-flowered sterile spikelets (fig. 1, B). At maturity the rachis breaks with a clean fracture at its joints into small segments, at the apex of each of which is attached a spikelet (pl. 5, B).

The spikelets are 7 mm wide and 11 mm long exclusive of the awns. On the exterior side of the spikelet at the base is a tuft of bristles which are 2 mm or less in length (pl. 5, *B*). At the base of the spikelet on the side facing the rachis is another tuft of bristles, which are 0.5 mm or less in length.

The rachis internodes are 2.5 to 3.0 mm long and glabrous except on the two lateral edges, which are fringed with bristles 0.5 to 3.0 mm long (pl. 5, *B*).

The glumes are glaucous, intermediate in shape between those of the two parents, 1.7 to 2.0 mm wide, 7 to 8 mm long exclusive of the awns, papillate except on the membranous portion near the edges, and each possesses five nerves, the two most prominent of which form keels. The rear keel is the primary or more prominent one of the two and is beset with individually arranged superasperites which are 2.0 mm or less in length. The primary keel of the glume terminates in a scabrid awn 2.0 to 2.5 cm long (pl. 5, *B*). The secondary or forward keel is beset with asperites 0.3 mm or less in length and terminates in a tooth 0.2 mm or less in length (pl. 5, *B*). Between the two keels is a very shallow, longitudinal depression, through the center of which passes laterally a conspicuous nerve which unites with the primary keel at the apex of the glume. The glume shoulders are intermediate between the two parents in width and have a sharp-pointed tooth (pl. 5, *B*).

The lemmas are 2.0 to 2.1 mm wide, 10 to 11 mm long exclusive of the awns, papillate on the exterior surfaces, and each has seven nerves, the middle one of which is partly keeled and terminates in a weakly purple-pigmented, scabrid awn 3.0 to 6.5 cm long. At the apex of the glume on the keel are several superasperites 1.3 mm or less in length (pl. 5, *B*). Some of the less conspicuous nerves located on the exposed portion of the lemma may also be scabrid.

The paleas are entire, not split. The lodicules are 0.5 mm wide and 1.5 mm long and are beset with hairs 0.42 mm long.

TRITICUM TIMOPHEEVI × HAYNALDIA VILLOSA

The plants of the hybrid *Triticum timopheevi* × *Haynaldia villosa* have an upright habit of growth and attain an average height at maturity of 149.86 cm. The culms are glaucous, glabrous, purple-pigmented, and hollow at a point 2.5 cm below the head. The nodes are puberulent with very short hairs.

The leaf blades are 8 to 11 mm wide and are covered on the upper and lower surfaces with hairs 2.5 mm or less in length. The edges of the blades are scabrid and possess hairs 3 mm or less in length. The leaf sheaths are purple-pigmented and glaucous. The upper leaf sheaths of the plant are usually devoid of hairs, while the lower ones may be sparsely covered with hairs. The overlapping lateral edge of each leaf sheath is beset with hairs 2 mm or less in length. The auricles are 1.2 mm long and are beset with several hairs 6 mm or less in length. The ligules project 1.3 mm or less from the point of attachment to the leaf.

The spikes are extremely fragile at maturity, 10 to 12 mm wide across the two-ranked face, 9 to 11.5 cm long exclusive of the awns, and composed of from thirty to thirty-five 3- or 4-flowered sterile spikelets (fig. 2, *B*). At maturity the rachis breaks with a clean

fracture at its joints into small segments, at the apex of each of which is attached a spikelet.

The spikelets are 4.0 to 5.5 mm wide and 16 to 19 mm long exclusive of awns. On the exterior side of the spikelets at the base is a tuft of bristles which are 1 mm or less in length (pl. 9, *E*). At the base of the spikelet on the side facing the rachis is a smaller tuft of bristles, which are 0.5 mm or less in length.

The rachis internodes are 3.0 to 3.5 mm long, sparsely puberulent, and papillate (pl. 9, *E*). The two lateral edges are fringed with bristles 1.5 mm or less in length (pl. 9, *E*).

The glumes are 2.0 to 2.5 mm wide, 8 mm long exclusive of beaks, glaucous, papillate on the exterior surfaces, and possess 5 to 6 nerves, the two most prominent of which are beset with superasperites 1.7 mm or less in length. The exterior surfaces of the glumes are usually devoid of hairs except on the margins of the glumes, which are partly pubescent (pl. 9, *E*). The awns on the glumes are purplish black, scabrid, and 5 cm or less in length. The glume shoulders are intermediate in width between those of the parents, elevated, and nondentate (pl. 9, *E*).

The lemmas are 2.5 to 3.0 mm wide, 12 to 14 mm long exclusive of the awns, papillate on the exterior surface, and partly pubescent on the upper exposed portions. The awns are purplish brown to purplish black. Superasperites usually are not present on the keels of the lemmas, but when present they are usually confined to the keel at the apex of the lemma.

The paleas are entire, not split. The lodicules are 0.8 mm wide and 1.9 mm long and are beset with hairs 0.3 mm long.

TRITICUM DICOCOIDES × HAYNALDIA VILLOSA

The plants of the hybrid *Triticum dicoccoides* × *Haynaldia villosa* have an upright habit of growth and attain an average height at maturity of 154.9 cm. The culms are glaucous, purple-pigmented, and hollow at a point 2.5 cm below the head. The nodes are puberulent.

The leaf blades are 7 to 8 mm wide and covered on the upper and lower surfaces with hairs 1 to 1.5 mm long. The edges of the blades are scabrid and possess hairs 2 to 3 mm long on the lower part. The leaf sheaths are purple-pigmented, glaucous, and glabrous on the outer surfaces, and are beset with hairs on one of the lateral edges. The auricles are 1.5 to 2 mm long with several hairs 5 mm or less in length. The ligules project 3 to 4 mm from the point of attachment to the leaf.

The spikes are extremely fragile, 8 to 10 mm wide across the two-ranked face, 10 to 12 cm long exclusive of the awns, and are composed of from thirty to sixty 3- to 5-flowered sterile spikelets (fig. 3, *B*). At maturity the rachis breaks with a clean fracture at its joints into small segments, at the apex of each of which is attached a spikelet (pl. 10, *B*; pl. 7, *B*; pl. 8, *B*).

The spikelets are 6 to 8 mm wide and 15 to 18 mm long exclusive of the awns (pl. 10, *B*). On the exterior side and center of the spikelet at the base is a prominent tuft of bristles which are 3 mm or less in length and which extend to the fringed edges of the rachis internode to which the spikelet is attached (pl. 6, *B*). At the base

of the spikelet on the side facing the rachis is a smaller tuft of bristles which are 1 mm or less in length (pl. 7, *B*).

The rachis internodes are 3 mm long, intermediate in shape between those of the two parents, glabrous on the convex and concave surfaces, and the edges are fringed with numerous bristles 5 mm or less in length (pl. 8, *B*).

The glumes are 2 mm wide, 10 to 12 mm long exclusive of the awns, glabrous, glaucous, white streaked with black pigment, papillate usually only on the prominent nerves, and each possesses five nerves, the two more prominent of which form keels. The rear one of the most prominent nerves is the stronger and forms the primary keel, which terminates in a black scabrid awn 35 to 38 mm long. The prominent nerve in the front forms a secondary keel and is scabrid. The primary keel is beset throughout its entire length with untufted superasperites 2.8 mm or less in length. These superasperites were present on the specimen illustrated in plate 10, *B*, but because of the laterally compressed spikelets they are completely obscured. Between the two prominent nerves is a longitudinal channel through which passes a conspicuous scabrid nerve which unites with a primary keel at the apex of the glume. The glume shoulders are intermediate in width between those of the parents, slightly elevated, and nontestate (pl. 10, *B*).

The lemmas are 2 mm wide, 12 to 14 mm long, papillate, slightly scabrous at their upper exposed surfaces, and possess nine inconspicuous nerves, the central one of which is partly keeled and terminates in a black scabrid awn 7 to 9 cm long. At the apex of the lemma are several superasperites 1 to 2 mm long (pl. 10, *B*).

The paleas are entire, not split. The lodicules are 0.9 mm wide and 1.8 mm long and are beset with hairs 0.65 mm long.

TRITICUM DICOCCUM × HAYNALDIA VILLOSA

The plants of the hybrid *Triticum dicoccum* × *Haynaldia villosa* have an upright habit of growth and attain an average height at maturity of 196.8 cm. The culms are hollow immediately below the head, glaucous, and slightly purple-pigmented. The nodes are very glaucous and puberulent.

The leaf blades are 8 to 10 mm wide and possess hairs on the upper and lower surfaces 1 to 1.5 mm long. The edges of the leaf blades are scabrid, and the lower edges of the blades are beset with hairs 0.5 to 1 mm long. The leaf sheaths are weakly glaucous, glabrous, and purple-streaked. The lateral edges of the sheaths are glabrous. The auricles are 2 mm long with a few hairs 3 mm or less in length. The ligules project 2 mm from the point of attachment to the leaves.

The spikes are very fragile, somewhat laterally compressed, 8 to 10 mm wide, 8 to 10 cm long exclusive of the awns and consist of from twenty to twenty-five 2- or 3-flowered sterile spikelets (fig. 4, *B*). At maturity the rachis breaks with a clean fracture at its joints into small segments at the apex of each of which is attached a spikelet (pl. 5, *E*).

The spikelets are 4.5 to 5.5 mm wide and 12 to 13 mm long exclusive of the awns (pl. 5, *E*). On the exterior side of the spikelet at the base is a distinct tuft of bristles which are 1 mm or less in length

(pl. 5, *E*). At the base of the spikelet on the side facing the rachis is also a tuft of hairs which are less than 0.5 mm long.

The rachis internodes are 4 to 5 mm long, nonglaucous, and glabrous except on the two lateral edges, which are fringed with numerous bristles 1 to 2 mm long (pl. 5, *E*).

The glumes are slightly glaucous, 4 mm wide, 11 to 12 mm long exclusive of the awns, papillate usually in greater abundance on the nerves, and possess 5 to 6 nerves, 2 or 3 of which may be scabrid. The two most prominent nerves form keels. The primary keel faces the rachis, possesses asperites on the lower edge and superasperites 1.5 mm or less in length on the upper edge, and terminates in an awn or beak 0.5 to 3.3 cm long (pl. 5, *E*). Between the prominent nerves is a longitudinal, shallow channel through which passes laterally 1 to 3 prominent nerves. The glume shoulders are intermediate in width between those of the parents and have a sharp-pointed tooth (pl. 5, *E*).

The lemmas are 3 mm wide, 12 to 13 mm long exclusive of the awns, papillate, slightly scabrid at the apex, and possess 7 to 8 nerves, the middle one of which forms an imperfect keel that terminates in a slightly purple-pigmented scabrid awn 8 to 8.7 cm long. The exposed portion of the keel at the apex of the lemma is usually beset with superasperites 0.8 to 1.0 mm long (pl. 5, *E*).

The paleas are entire, not split. The lodicules are 1 mm wide and 2 mm long and are beset with hairs 0.4 mm long.

TRITICUM DURUM \times HAYNALDIA VILLOSA

The plants of the hybrid *Triticum durum* \times *Haynaldia villosa* have an upright habit of growth and attain an average height at maturity of 152.4 cm. The culms are glaucous, purple-pigmented, and hollow at a point 2.5 cm below the head (pl. 3, *E*). The nodes are glaucous and glabrous.

The leaf blades are 10 to 11 mm wide and covered on the upper and lower surfaces with hairs 1 to 1.25 mm long. Hairs 1 to 1.25 mm long are present also on the edges of the leaves for a distance of 6 to 7 cm upward from the base. The edges of the leaf blades are scabrid. The leaf sheaths are glaucous, glabrous, and purple-pigmented and are devoid of hairs on the lateral edge. The auricles are 2.5 to 3 mm long and possess hairs 4 mm or less in length. The ligules project 3 mm from the point of attachment to the leaf.

The spikes are extremely fragile, 10 to 12 mm wide across the two-ranked face, 8 to 12 cm long, and composed of from twenty to thirty 3- or 4-flowered sterile spikelets (fig. 5, *B*). At maturity the rachis breaks with a clean fracture at its joints into small segments, at the apex of each of which is attached a spikelet (pl. 11, *E*).

The spikelets are 7 to 9 mm wide and 12 to 14 mm long exclusive of the awns (pl. 11, *E*). On the exterior side of the spikelet at the base is a collar of bristles, the longest of which do not exceed 1.5 mm (pl. 11, *E*). At the base of the spikelet on the side facing the rachis is a tuft of slender bristles 0.4 mm or less in length.

The rachis internodes are 4 mm long, glaucous, and glabrous, and the edges are fringed with numerous bristles, the longest of which does not exceed 2 mm (pl. 11, *E*).

The glumes are papillate, glaucous, 2 mm wide, 9 to 10 mm long exclusive of the awns, and possess four prominent nerves, the most conspicuous of which forms the keel and terminates in a beak or short awn 10 to 26 mm long. The keel has short asperities except near the apex, where they take the shape of long bristlelike structures about 1 mm long. The glume shoulders are intermediate in width between those of the parents, elevated, and nondentate (pl. 11, *E'*).

The lemma is papillate, glaucous, slightly asperous at its upper exposed surface, 2.5 mm wide and 11 to 12 mm long, and possesses 9 to 10 nerves, the most prominent of which terminates in a slightly purple-pigmented scabrous awn 3 to 7 cm long. At the apex of the lemma on the keel are usually several conspicuous trichomes 1 mm or less in length (pl. 11, *E*).

The paleas are entire, not split. The lodicules are 1 mm wide and 1.8 mm long and are beset with hairs 0.3 mm long.

TRITICUM POLONICUM × HAYNALDIA VILLOSA

The plants of the hybrid *Triticum polonicum* × *Haynaldia villosa* have an upright habit of growth and attain an average height at maturity of 133.3 cm. The culms are glaucous, hollow, and purple-pigmented. The nodes are usually glabrous, though rarely they are sparsely puberulent and slightly glaucous.

The leaf blades are 10 to 12 mm wide and are beset on the upper and lower surfaces with a few hairs 0.5 to 1 mm long. The margins of the leaf blades are scabrid and beset with hairs 2 mm or less in length for a distance of several centimeters from the base of the leaf. The leaf sheaths are usually glaucous, streaked with purple pigment, and glabrous on the surfaces and edges. Sometimes on the lower leaf sheaths one lateral edge may be abundantly covered with hairs 1.5 to 2 mm long. The auricles are 2 to 3 mm long, with many hairs 5 mm or less in length. The ligules project 2.5 to 3 mm from the point of attachment to the leaf.

The spikes are fragile, 10 to 12 mm wide across the two-ranked face, 12 to 14 cm long exclusive of the awns, and consist of from twenty-five to thirty 2- to 5-flowered sterile spikelets (fig. 6, *B*).

The spikelets are 9 to 13 mm wide and 18 to 20 mm long exclusive of awns (pl. 9, *B*). On the exterior side of the spikelet at the base is a prominent tuft of bristles which are 0.5 to 2 mm long (pl. 9, *B*). At the base of the spikelet on the side facing the rachis is a tuft of bristles, which are 0.5 mm or less in length and extend to the right and left, joining the lateral fringes of bristles on the edges of the rachis internode to which the spikelet is attached.

The rachis internodes are 5 to 6 mm long, glaucous, and glabrous except on their lateral edges, which may be entirely fringed with bristles 0.5 to 2.5 mm long, the shorter bristles being at or near the base and increasing progressively as they approach the apex. Sometimes the lower edge of the rachis internode for a distance of 1 or 2 mm may be entirely devoid of bristles (pl. 9, *B*).

The glumes are partly glaucous, papillate, 3 mm wide, 17 to 19 mm long exclusive of the awns, and possess 9 to 10 nerves, the most prominent of which forms a sharp scabrid keel that terminates in a scabrid awn 2 to 3 cm long (pl. 9, *B*). The keel throughout its entire length

is beset with asperites. Besides the keel 1 or 2 other prominent nerves may be scabrid. The apex and membranous portion of the glume are abundantly puberulent or pubescent (pl. 9, *B*). No superasperites appear on the keel. The glume shoulders are intermediate in width between those of the parents, elevated, and nondentate (pl. 9, *B*).

The lemmas are 3 to 3.5 mm wide, 17 to 19 mm long exclusive of awns, papillate, puberulent, and pubescent on the edges and upper exposed portions (pl. 9, *B*). The lemmas also have 8 to 9 nerves, the middle one of which forms a partial keel that is beset with a few short superasperites and terminates in a purplish-black scabrid awn 2 to 6.5 mm long (pl. 9, *B*). Some of the nerves on the exposed portion of the lemmas may be scabrid also.

The paleas are entire, not split. The lodicules are 1 mm wide, 2.3 mm long and are beset with hairs 0.58 mm long.

TRITICUM TURGIDUM \times HAYNALDIA VILLOSA

The plants of the hybrid *Triticum turgidum* \times *Haynaldia villosa* have an upright habit of growth and attain an average height at maturity of 177.8 cm. Each culm is hollow with a small cavity at a point 2.5 cm below the head, glaucous, and purple-pigmented. The nodes are glabrous.

The leaf blades are 7 to 9 mm wide and puberulent on both the upper and lower surfaces. The edges of the blades are scabrid and possess a few hairs 1 to 2 mm long on the lower part of the blades. The auricles are 2 mm long with a few hairs 3 mm or less in length. The leaf sheaths are purple-pigmented, glabrous, slightly glaucous on the surfaces, and glabrous on the overlapping edges. The ligules project 3 to 4 mm from the point of attachment to the leaf.

The spikes may be simple or branched, very fragile, 9 to 40 mm wide across the two-ranked face, 12 to 13 cm long exclusive of the awns, and consist of from forty to sixty-five 2- to 4-flowered spikelets (fig. 7, *B*).

The spikelets are 8 to 9 mm wide and 17 to 18 mm long exclusive of the awns (pl. 11, *B*). Most of the spikelets are completely sterile, although a few kernels were obtained that are longer than either of the parents (pl. 2, *F*). On the exterior side of the spikelet at the base is a tuft of bristles which are 2.5 or less in length and extend to the two lateral edges of the rachis internode to which the spikelet is attached (pl. 11, *B*). At the base of the spikelet on the side facing the rachis is another tuft of bristles which are 1 mm or less in length.

The rachis internodes are intermediate in shape between those of the parents, 3 to 3.5 mm long, and glabrous on the surfaces, and on each of the two lateral edges is a fringe of bristles 0.5 to 3.5 mm long, the longest being located at the apex of the rachis internode and the shortest at the base (pl. 11, *B*).

The glumes are 4 mm wide, 7 to 9 mm long exclusive of the awns, papillate on the exterior surface except on the membranous portion near the edges, and each possesses four nerves, the two most prominent of which form keels. The rear one of the two most prominent nerves is the stronger and forms the primary keel, at the apex of which is a black awn 1.5 to 2.5 cm long (pl. 11, *B*). The glume

shoulders are intermediate in width between those of the parents, elevated, and nondentate (pl. 11, *B*).

The prominent nerve nearest the front forms a secondary keel. The two keels of the glumes of this hybrid are beset with untufted superasperites which have a maximum length of 8.3 mm (pl. 11, *B*; pl. 12, *B*). Between the two prominent nerves is a shallow, longitudinal channel through which passes a conspicuous nerve which unites with the primary keel at the apex of the glume.

The lemmas are 3 to 4 mm wide and 10 to 13 mm long, papillate, and terminate in a purplish black scabrid awn 5 to 9 cm long. At the apex of the lemma on the keel are several untufted superasperites 1.7 mm or less in length (pl. 1, *E*; pl. 11, *B*; and pl. 12, *E*). Also a few asperites may be found on the exposed portion of the lemma, usually on the nerves.

The paleas are entire, not split. The lodicules are 0.9 mm wide and 1.8 mm long and are beset with hairs 0.5 mm long.

SECALE FRAGILE × HAYNALDIA VILLOSA

The plants of the hybrid *Secale fragile* × *Haynaldia villosa* have an upright habit of growth and attain an average height at maturity of 106.7 cm. The culms are hollow, purple-pigmented, and glaucous, and the peduncle below the head for several centimeters is covered with hairs which are less than 1 mm in length (pl. 2, *B*; pl. 3, *H*). The nodes are glabrous.

The leaf blades are 5 to 6 mm wide and possess on the upper and lower surfaces an abundance of hairs 1 to 2 mm long. The edges of the leaf blades are scabrid and beset with hairs 1 to 3.0 mm long. Just above the ligule on the leaf is a collar of hairs slightly shorter and fewer in number than those of the *Secale* parent (pl. 4, *B*). The exterior surfaces of the lower leaf sheaths are hairy or puberulent on the exterior surfaces and glabrous on the edges. The leaf sheaths are also weakly glaucous, and slightly purple-pigmented, and the overlapping lateral edges are devoid of hairs. The auricles are 2 mm long with several hairs 4 mm or less in length. The ligules project 3 mm from the point of attachment to the leaves.

The spikes are extremely fragile, 8 to 9 mm wide across the two-ranked face, 10 to 12 cm long exclusive of the awns, and consist of from thirty to thirty-eight 3- or 4-flowered sterile spikelets (fig. 8, *B*). At maturity the rachis breaks with a clean fracture at its joints into small segments, at the apex of each of which is attached a spikelet (pl. 10, *E*).

The spikelets are 5 to 7 mm wide and 16 to 18 mm long exclusive of the awns (pl. 10, *E*). On the exterior side of the spikelet at the base is a tuft of short bristles 0.35 mm or less in length (pl. 10, *E*). At the base of the spikelet on the side facing the rachis is a tuft of short bristles which are 0.5 mm or less in length.

The rachis internodes are 3 mm long and usually glabrous on the exterior surface, though occasionally there may be an irregular distribution of extremely short bristles (pl. 10, *E*). The edges of the rachis internodes are fringed with numerous bristles 3.5 mm or less in length.

The glumes are slightly glaucous, 1 mm wide, 8 to 10 mm long exclusive of the awns, papillate except on the membranous portions

near the edges, and possess two prominent scabrid nerves which form keels and are separated by a boat-shaped depression about 0.75 mm wide at the center. The two keels converge at the base and apex. At the apex of each glume is a long scabrous awn 3 to 5.5 cm long (pl. 10, *E*). The primary or more prominent keel, however, possesses abundant superasperites 1.9 mm or less in length, while the secondary keel usually is beset with asperites. The glume shoulders are intermediate in width between those of the parents, slightly elevated to oblique, and nondentate (pl. 10, *E*).

The lemmas are 2.5 mm wide, 12 to 14 mm long exclusive of the awns, and papillate on the exterior surface, and each has five nerves, the middle one of which is keeled and terminates in a purple, scabrid awn 5 to 7 cm long (pl. 10, *E*). The keels of the lemmas throughout their entire length are beset with slender superasperites 1.4 mm or less in length. The exposed surface of the lemma and the margins usually are beset with reduced spur asperites (pl. 10, *E*). The edges of the lemma are beset with thin hairs 0.75 mm or less in length.

The paleas are entire, not split. The lodicules are 1 mm wide and 2.9 mm long and are beset with hairs 0.66 mm long.

DISCUSSION

Hybrid seeds were obtained and the F_1 plants matured from the crosses of *Haynaldia villosa* on *Triticum aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. turgidum*, *T. polonicum*, and *Secale fragile*. Attempts to cross *T. vulgare*, *T. spelta*, *T. compactum*, and *S. cereale* with *H. villosa* have met with failure. Several varieties of *T. vulgare* when pollinated with pollen from *H. villosa* produced enlarged structures which were devoid either of embryos or of endosperms. One hybrid seed of the cross *T. vulgare* var. C. I. 6223 \times *H. villosa* germinated but died before the formation of the third leaf.

From Strampelli's successful cross of *T. vulgare* with *T. villosum* (*Haynaldia*), as reported by Raineri (6), and a similar cross with another, *T. spelta* \times *T. villosum*, by Tschermak (8, 9), it is probable that these investigators may have had a variety of *H. villosa* different from that used by the writer. The writer has been unable to cross *T. vulgare* and *T. spelta* with *H. villosa*.

The form of *Haynaldia villosa* used by the writer was procured from Russia, grew normally to a height of 120 to 130 cm in a greenhouse, and set seed satisfactorily under these conditions and out of doors. Another form of *H. villosa* introduced from Denmark under the name *Agropyron villosum* grew to a height of only 40 cm and showed abnormal anther dehiscence, which resulted in almost complete sterility of the plants. Because of the difficulty in procuring pollen from the latter form no attempts were made to make hybrids with it.

Special attention was devoted to a study of the trichomes because of the variation of their shape, length, and thickness.

The lodicule is an interesting organ which has received little attention in morphological studies of the grasses. Preliminary studies of the lodicules of *Triticum*, *Secale*, *Aegilops*, *Haynaldia*, and *Agropyron* species indicate that these organs have certain characteristics that may be used in the identification of species and

genera. There are differences in shape, dimensions, and marginal indentations, and in the length and distribution of the hairs on the exterior surfaces. Plate 13 shows the lodicules of *T. aegilopoides*, *T. polonicum*, *S. fragile*, and *H. villosa* and of F_1 hybrids of the first three species with *Haynaldia*.

The various species used in the production of the hybrids described fall into two chromosome groups. *Triticum aegilopoides*, *Secale fragile*, and *Haynaldia villosa* are diploid species, while *T. timopheevi*, *T. dicoccoides*, *T. dicoccum*, *T. polonicum*, *T. durum*, and *T. turgidum* are tetraploid species. Although *H. villosa*, *S. fragile*, and *T. aegilopoides* each have seven haploid chromosomes, when *H. villosa* was crossed with *S. fragile* and *T. aegilopoides* the F_1 hybrids were completely self-sterile. This has particular significance, especially in view of the fact that in the latter hybrid a maximum of five pairs of chromosomes were observed during meiosis. With the exception of *T. turgidum* var. Alaska, all of the tetraploid species referred to above when crossed with *H. villosa*, a diploid species, produced hybrids that were completely self-sterile. The cross *T. turgidum* var. Alaska \times *H. villosa* is the only F_1 hybrid among all of the crosses made that showed any fertility, and this produced only an average seed set of 3.8 percent with a maximum seed set of 11.1 percent for a single plant. The F_2 plants of this cross produced an average seed set of 29.7 percent with a maximum seed set of 58.8 percent for a single plant, and the F_3 plants produced an average seed set of 58.5 percent with a maximum seed set of 76.9 percent for a single plant. No apparent segregation occurred in the F_2 and subsequent generations of the cross *T. turgidum* \times *H. villosa*, the F_1 type remaining fixed in its morphological characters. In figure 9 are shown F_2 spikes.

In general, the F_1 hybrids of the above crosses resemble the *Triticum* or *Secale* parent, although a critical study indicates that the majority of the characters of the F_1 hybrids are intermediate between those of the parents involved. Some characters of the F_1 , however, show a decided increase over those of either parent, while others show a dominance of one or the other parent. For convenience in comparison 52 characters showing characteristics of the parents and F_1 hybrids are listed in table 2. These are discussed in the following paragraphs, which are numbered to correspond with the table.

1. The F_1 hybrids of *Triticum timopheevi*, *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. turgidum*, and *T. polonicum* with *Haynaldia villosa* are greater in stature than either parent, while those of *T. aegilopoides* and *Secale fragile* with *H. villosa* are somewhat intermediate to parental stature.

2. The culms of *Haynaldia villosa* are hollow at a point 2.5 cm below the head and possess large cavities. *Triticum polonicum* and *Secale fragile* have hollow culms with small cavities. When these are crossed with *H. villosa* the F_1 plants have cavities intermediate between those of the parents. *T. aegilopoides*, *T. dicoccoides*, *T. dicoccum*, and *T. turgidum* all have solid culms at a point 2.5 cm below the head. When these are crossed with *H. villosa* the F_1 hybrids have culms with cavities similar in size to those of the *Haynaldia* parent. Crosses between *T. timopheevi* and *H. villosa*, both of which have large culm cavities, produced F_1 hybrids with culm cavities larger than those of either parent. Crosses between *T. durum* and *H. villosa*, with culm cavities similar in size, produced F_1 plants with culm cavities not appreciably different in size from those of either parent. Cross sections of the culms 2.5 cm below the spike of the parents and F_1 hybrids of the crosses *T. aegilopoides*, *T. durum*, and *S. fragile* with *H. villosa* are shown in plate 3.

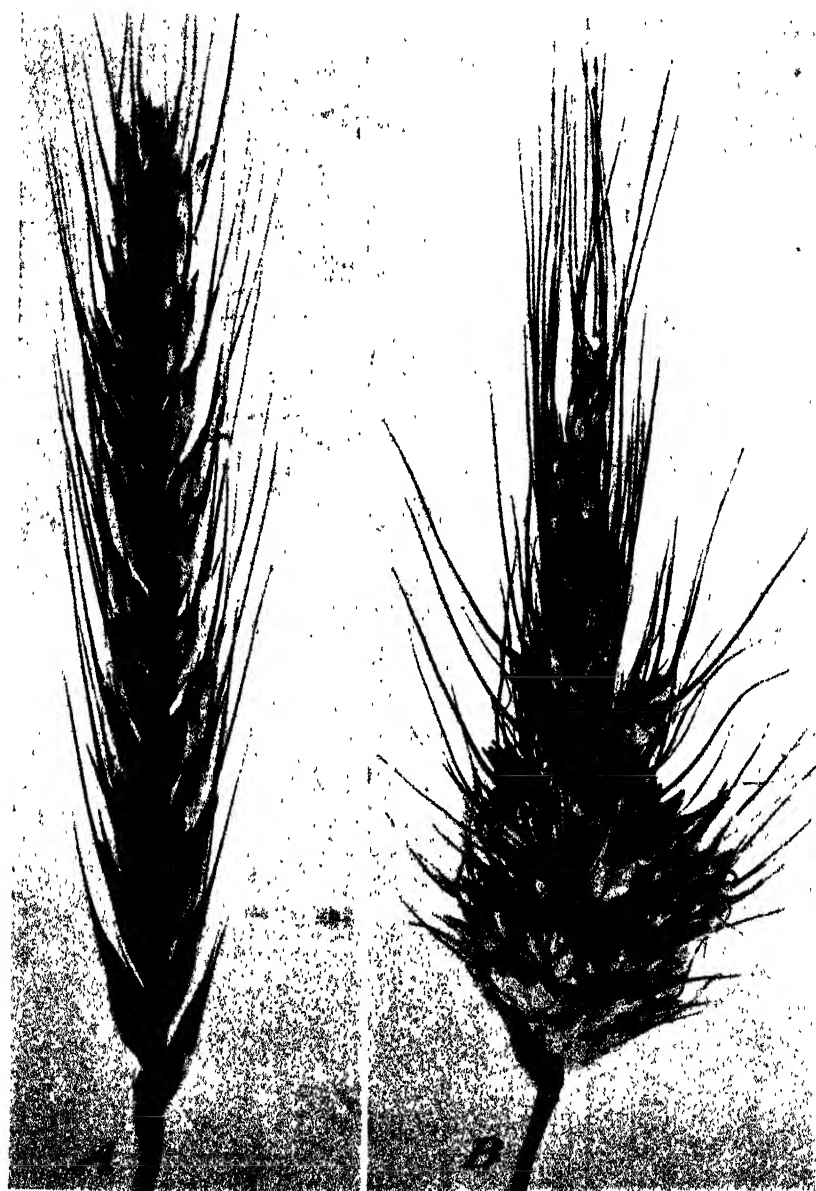


FIGURE 9.— F_2 spikes of *Triticum turgidum* \times *Haynaldia villosa*: A, Simple; B, ramified. Natural size.

TABLE 2.—Some characters of *Triticum*, *Secale fragile*, and *Haynaldia villosa* and their expression in the F_1 hybrids resulting from the crossing of *Triticum* and *Secale* species with *H. villosa*.[Plus (+) denotes presence, and minus (-) absence, of character ¹ named]

Characters ²	<i>T. aestivoides</i> × <i>H. villosa</i>		<i>T. timopheevi</i> × <i>H. villosa</i>		<i>T. dicoccoides</i> × <i>H. villosa</i>		<i>T. dicoccum</i> × <i>H. villosa</i>	
	<i>Triticum</i> parent	F_1 hybrid	<i>Triticum</i> parent	F_1 hybrid	<i>Triticum</i> parent	F_1 hybrid	<i>Triticum</i> parent	F_1 hybrid
1. Stature of plant (average).....	124.5	116.8	142.2	149.9	76.2	154.9	98.6	196.3
2. Solidity of culm 1 inch below head.....	Hollow	Hollow	Hollow	Hollow	Solid	Hollow	Solid	Hollow
3. Anthocyanin in culm.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
4. Pubescence of culm.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
5. Glauconess of culm.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
6. Pubescence of culm nodes.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
7. Width of leaf blades (average).....	7	6.5	8	9.5	7	7.5	11	9
8. Pubescence of leaf-blade margins.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
9. Scabrousness of leaf-blade margins.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
10. Pubescence of leaf sheaths.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
11. Glauconess of leaf sheaths.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
12. Anthocyanin in leaf sheaths.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
13. Pubescence of leaf-sheath margins.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
14. Length of auricles (maximum).....	2	1.5	1	1.2	1	2	2	2
15. Length of hairs on auricles (maximum).....	4	3	5	6	1.5	4	0.7	3
16. Length of ligules (maximum).....	3	1.5	1	1.3	3	4	2	2
17. Width of spike (average).....	12	7	13.5	11	8.5	9	9	9
18. Length of spike (average).....	8	11	10.2	10.2	8	11	8	9
19. Spikelets.....	20-33 (26)	30-40 (38)	18-21 (19)	30-35 (33)	16-18 (17)	30-50 (45)	16-24 (20)	20-25 (23)
20. Width of spikelets (average).....	5.5	7	7.5	4.8	4.5	7	6.5	6.5
21. Length of spikelets (average).....	13.5	10	17	17.5	16	16.5	11	12.5
22. Branching of rachis.....	Simple	Simple	Simple	Simple	Simple	Simple	Simple	Simple
23. Fragility of rachis.....	Very fragile	Very fragile	Nonfragile	Very fragile	Very fragile	Very fragile	Very fragile	Very fragile
24. Shape of rachis internodes.....	Obovate	Intermediate	Blunt-cuneate	Intermediate	Oblong	Oblong	Oblong	Intermediate
25. Length of rachis internodes.....	2.5	2.7	2	3.3	4	3	2	4.5
26. Arrangement of bristles on rachis.....	Tufted	Non tufted	Non tufted	Non tufted	Non tufted	Non tufted	Non tufted	Non tufted
27. Width of glumes (average).....	2	1.8	2.5	2.3	2	2	2.5	4
28. Length of glumes (average).....	5.5	7.5	9	8	12.5	11	9.5	11.5
29. Length of beak on glumes (maximum).....	30	25	5	50	2	38	0.5	33
30. Shape of shoulder on glumes.....	Elevated-rounded	Apiculate	Apiculate	Apiculate	Apiculate	Rounded	Acute to apiculate	Rounded
31. Size of papillae on glumes.....	Large	Medium	(-)	Medium	Small	Medium	Small	Medium
32. Canalisation of glumes.....	(+)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
33. Carination of glumes.....	Bicardate	Bicardate	Uncardate	Bicardate	Uncardate	Bicardate	Uncardate	Bicardate
34. Glauconess of glumes.....	(+)	(+)	(+)	(+)	(-)	(+)	(-)	(+)

TABLE 2.—Some characters of seven species of *Triticum*, *Secale fragile*, and *Haynaldia villosa* and their expression in the F_1 hybrids resulting from the crossing of *Triticum* and *Secale* species with *H. villosa*—Continued
[Plus (+) denotes presence, and minus (—) absence, of character¹ named]

Characters ¹	<i>T. durum</i> × <i>H. villosa</i>		<i>T. polonicum</i> × <i>H. villosa</i>		<i>T. turgidum</i> × <i>H. villosa</i>		<i>S. fragile</i> × <i>H. villosa</i>	
	<i>Triticum</i> parent	F_1 hybrid	<i>Triticum</i> parent	F_1 hybrid	<i>Triticum</i> parent	F_1 hybrid	<i>Secale</i> parent	F_1 hybrid
1. Stature of plant (average).....	124.5	124.4	124.5	133.3	127	177.8	76	106.7
2. Solidity of culm 1 inch below head.....	Hollow	Hollow	Small cavity	Hollow (int.)	Solid	Hollow	Hollow	Hollow
3. Anthocyanin in culm.....	(+)	(+)	(—)	(+)	(—)	(+)	(+)	(+)
4. Pubescence of culm.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
5. Pubescence of culm nodes.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
6. Pubescence of leaf blades (average).....	9	10.5	30	11	16.5	8	6.6	5.5
7. Width of leaf blades (average).....	mm	mm	mm	mm	mm	mm	mm	mm
8. Pubescence of leaf-blade margins.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
9. Pubescence of leaf-blade margins.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
10. Pubescence of leaf sheaths.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
11. Pubescence of leaf sheaths.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
12. Anthocyanin in leaf sheaths.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
13. Pubescence of leaf-sheath margins.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
14. Length of auricles (maximum).....	mm	mm	mm	mm	mm	mm	mm	mm
15. Length of hairs on auricles (maximum).....	do.	do.	do.	do.	do.	do.	do.	do.
16. Length of ligules (maximum).....	do.	do.	do.	do.	do.	do.	do.	do.
17. Width of spike (average).....	do.	do.	do.	do.	do.	do.	do.	do.
18. Length of spike (average).....	cm	cm	cm	cm	cm	cm	cm	cm
19. Spikelets.....	8	10	12	13	10	12	9.5	11
20. Width of spikelets (average).....	mm	mm	mm	mm	mm	mm	mm	mm
21. Length of spikelets (average).....	5.5	5.5	18-22 (20)	25-30 (27)	30-40 (55)	40-45 (47)	26-36 (31)	30-38 (34)
22. Branching of rachis.....	Simple	Simple	Simple	Simple	Branched	Branched	Simple	Simple
23. Fragility of rachis.....	Very fragile	Very fragile	Nonfragile	Nonfragile	Nonfragile	Very fragile	Simple	Very fragile
24. Shape of rachis internodes.....	Obovate	Oblong	Oblong	Intermediate	Oblong	Intermediate	Oblong	Intermediate
25. Length of rachis internodes.....	mm	mm	mm	mm	mm	mm	mm	mm
26. Arrangement of bristles on rachis.....	Tufted	Nontufted	Nontufted	Nontufted	Nontufted	Nontufted	Nontufted	Nontufted
27. Width of glumes (average).....	mm	mm	mm	mm	mm	mm	mm	mm
28. Length of glumes (average).....	5.5	5.5	22	18	7.5	8	0.5	1
29. Length of beak on glumes (maximum).....	do.	do.	do.	do.	do.	do.	do.	do.
30. Shape of shoulder on glumes.....	Elevated-rounded	Elevated (int.)	Apiculate	Rounded (int.)	Oblong	Rounded	60	55
31. Size of papillae on glumes.....	Large	Medium	Small	Medium	Small	Medium	Small	Medium
32. Carination of glumes.....	Bicarinata	Bicarinata	Uncarinate	Bicarinata	Uncarinate	Bicarinata	Uncarinate	Bicarinata
33. Carination of glumes.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
34. Glauconousness of glumes.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
35. Pubescence of glumes.....	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
36. Width of lemma (average).....	mm	mm	mm	mm	mm	mm	mm	mm

37. Length of lemma (average).....mm.	12	8.5	11.5	24	18	8	11.5	16	13.
38. Length of awn on lemma (maximum).....mm.	4.5	16	7	12	6.5	11	9	12	7.
39. Pigment in awn on lemma.....	Purple.	Yellow.	Weak purple.	Black.	Purplish black.	Black.	Purplish black.	Purple.	Purple.
40. Size of papillae on lemma.....	Large	Small	Medium	Small	Medium	Small	Medium	Small	Medium.
41. Length of trichomes on glume keels.....	Long	Short	Intermediate	Short	Intermediate	Short	Intermediate	Short	Intermediate.
42. Arrangement of trichomes on glume keels.....	Tufted	Short	Intermediate	Nontufted	Nontufted	Nontufted	Nontufted	Nontufted	Nontufted.
43. Length of trichomes on lemma keels.....	Long	Short	Intermediate	Short	Intermediate	Short	Intermediate	Short	Intermediate.
44. Arrangement of trichomes on lemma keels.....	Tufted	Short	Intermediate	Nontufted	Nontufted	Nontufted	Nontufted	Nontufted	Nontufted.
45. Length of bristles at base of spikelet on exterior side (maximum), mm.	0.5	2	1.5	2.5	2	2	1	0.2	0.35.
46. Bristles at base of spikelet on exterior side, number.	Few	Many	Intermediate	Many	Intermediate	Many	Intermediate	Several	Intermediate.
47. Length of bristles at base of spikelet on side facing rachis (maximum), mm.	0.3	0.35	0.55	0.3	0.5	0.5	0.5	0.35	0.5.
48. Bristles at base of spikelet on side facing rachis, number.	Few	Many	Intermediate	Many	Intermediate	Many	Intermediate	Many	Intermediate.
49. Width of lodicules (maximum).....mm.	0.9	0.9	1	1.0	1.0	0.7	0.9	1.1	1.
50. Length of lodicules (maximum).....mm.	2	1.3	1.8	1.6	2.3	1.1	1.8	2.9	2.0.
51. Length of hairs on lodicules (maximum), mm.	0.05	0.5	0.3	1.35	0.58	0.7	0.5	1.25	0.66.
52. Self-fertility of plant.....	Fully fertile.	Fully fertile.	Infertile.	Fully fertile.	Infertile.	Fully fertile.	Weakly fertile.	Fully fertile.	Infertile.

Int.=Intermediate.

Average and maximum denote, respectively, average and maximum of several measurements.

3. Anthocyanin formation in the stems is dominant over the absence of this function. The culms of *Triticum aegilopoides*, *T. dicoccoides*, and *Secale fragile* possess anthocyanin pigment. *T. timopheevi*, *T. dicoccum*, *T. polonicum*, *T. durum*, and *T. turgidum* have culms devoid of anthocyanin pigment. When these latter are crossed with *Haynaldia villosa*, which has anthocyanin pigment in its stems, the F₁ hybrid plants possess anthocyanin pigment.

4. Papillate and pubescent peduncles are dominant over glabrous peduncles. All of the species here reported on, with the exception of *Secale fragile* and *Triticum timopheevi*, have glabrous peduncles. *T. timopheevi* has papillate peduncles and when crossed with *Haynaldia villosa* with glabrous peduncles produced F₁ hybrids with papillate peduncles. The papillae of this hybrid are fewer in number and smaller in size, however, than those of the *T. timopheevi* parent. The peduncles immediately below the spike of *S. fragile* are abundantly pubescent. When this species is crossed with *H. villosa* the resultant hybrids have pubescent peduncles. The hairs of the hybrids, however, are greater in number but slightly shorter than those of the pubescent parent (pl. 2, A, B, C).

5. Glaucous culm is dominant over nonglaucous culm. *Triticum timopheevi*, *T. dicoccoides*, *T. durum*, *T. turgidum*, *T. polonicum*, *Secale fragile*, and *Haynaldia villosa* all have glaucous culms. The culms of *T. aegilopoides* and *T. dicoccum* are not glaucous. When these latter two are crossed with *H. villosa* the F₁ hybrids have glaucous culms.

6. Considerable variation occurs in the length, number, and distribution of hairs on the nodes of the parents and hybrids. In some species considerable variation in hairiness exists even on the separate nodes of the same culm. When *Triticum aegilopoides* and *T. dicoccoides*, with pubescent nodes, are crossed with *Haynaldia villosa*, with glabrous nodes, the F₁ hybrids have nodes with intermediate hair development. *T. timopheevi*, *T. dicoccum*, and *T. polonicum*, with puberulent nodes, when crossed with *H. villosa* produced hybrids with nodal hairs usually shorter and fewer than those possessed by the puberulent parent. *Secale fragile*, with puberulent nodes, when crossed with *H. villosa* produced F₁ hybrids having glabrous nodes. *T. durum* and *T. turgidum* have glabrous nodes.

7. Considerable variation in width of the leaf blades exists among the parents and F₁ hybrids of the various crosses. *Triticum aegilopoides*, with leaves 5 mm in width, represents the narrowest, while *T. polonicum*, with leaves 20 mm in width, represents the widest. *T. timopheevi*, *T. dicoccoides*, *T. durum*, *T. dicoccum*, *T. polonicum*, and *T. turgidum* are all equal to or greater than *Haynaldia villosa* in leaf width. When *T. timopheevi*, *T. dicoccoides*, and *T. durum* are crossed with *Haynaldia*, F₁ hybrids are produced with leaves greater in width than those of either parent. *T. dicoccum*, *T. polonicum*, and *T. turgidum*, when crossed with *Haynaldia*, produced F₁ hybrids with leaves intermediate in width between those of the parents. *T. aegilopoides* and *Secale fragile* are less in leaf width than *Haynaldia*, and hybrids of the former with *Haynaldia* have leaf blades intermediate in width between the parents, while hybrids of the latter with *Haynaldia* have leaf blades less in width than either.

8. Pubescent leaf blade margins are dominant over glabrous leaf blade margins. *Triticum aegilopoides*, *T. timopheevi*, *T. polonicum*, *Secale fragile*, and *Haynaldia villosa* have leaf blades with pubescent margins. *T. dicoccoides*, *T. dicoccum*, *T. turgidum*, and *T. durum* have leaf-blade margins devoid of pubescence. When the four latter are crossed with *Haynaldia* the F₁ hybrids possess pubescence on leaf-blade margins.

9. Scabrid leaf-blade margins are dominant over their absence. *Triticum aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. polonicum*, *Secale fragile*, and *Haynaldia villosa* have scabrid leaf-blade margins. *T. turgidum* has nonscabrid leaf-blade margins. When the latter is crossed with *Haynaldia*, F₁ plants with scabrid leaf-blade margins result.

10. Pubescent leaf sheaths are dominant over glabrous leaf sheaths. *Triticum dicoccoides*, *T. dicoccum*, *T. durum*, *T. polonicum*, *T. turgidum*, and *Haynaldia villosa* have glabrous leaf sheaths. *T. aegilopoides*, *T. timopheevi*, and *Secale fragile* have pubescent leaf sheaths. When these are crossed with *Haynaldia* the F₁ plants possess pubescence on the leaf sheaths.

11. Glaucous leaf sheaths are dominant over absence of this character. The leaf sheaths of *Triticum aegilopoides* and *T. dicoccoides* are nonglaucous. *T. timopheevi*, *T. dicoccum*, *T. durum*, *T. polonicum*, *T. turgidum*, *Secale fragile*,

and *Haynaldia villosa* have glaucous leaf sheaths. *T. aegilopoides* and *T. dicoccoides*, when crossed with *Haynaldia*, produce hybrids with glaucous leaf sheaths.

12. Anthocyanin pigmentation of the leaf sheaths is dominant over its absence. *Triticum dicoccoides*, *Secale fragile*, and *Haynaldia villosa* have anthocyanin-pigmented leaf sheaths. *T. aegilopoides*, *T. timopheevi*, *T. dicoccum*, *T. durum*, *T. polonicum*, and *T. turgidum* are without anthocyanin pigmentation. When these are crossed with *Haynaldia* the F_1 hybrids have anthocyanin-pigmented leaf sheaths.

13. Pubescence of overlapping leaf-sheath margin is dominant over its absence. *Triticum dicoccum*, *T. durum*, *T. polonicum*, *T. turgidum*, *Secale fragile*, and *Haynaldia villosa* all have overlapping leaf-sheath margins devoid of pubescence. *T. aegilopoides*, *T. timopheevi*, and *T. dicoccoides* have pubescent overlapping leaf-sheath margins. When the three latter are crossed with *Haynaldia* the F_1 hybrids all possess pubescence on the overlapping leaf-sheath margins.

14. The length of the auricle varies among the different species and hybrids. *Triticum aegilopoides* and *T. timopheevi*, with short auricles, crossed with *Haynaldia villosa*, possessing longer auricles, produced F_1 hybrids somewhat intermediate in auricle length between those of the two parents. When *T. dicoccoides*, *T. turgidum*, and *Secale fragile* are crossed with *H. villosa* the F_1 hybrids are similar to the *Haynaldia* parent in auricle length. *T. dicoccum* and *H. villosa* are the same in auricle length, and their F_1 has auricles of the same length as the parents. Crosses of *T. durum* and *T. polonicum* with *Haynaldia* produce F_1 hybrids with auricles greater in length than those of either parent.

15. With the exception of *Triticum polonicum* and *T. durum*, all of the species used in the crosses here reported have hairs on the auricles, these ranging from 0.25 to 6.0 mm in length. *T. aegilopoides*, *T. dicoccum*, and *T. turgidum* crossed with *Haynaldia villosa* produced hybrids having auricle hairs intermediate between those of the two parents. Crosses of *T. timopheevi* and *T. dicoccoides* with *Haynaldia* produced hybrids with auricle hairs greater in length than those of the parents. *T. polonicum*, without auricle hairs, crossed with *Haynaldia* produced an F_1 with auricle hairs greater than those of the *Haynaldia* parent. *T. durum*, without auricle hairs, crossed with *Haynaldia* produced an F_1 having auricle hairs similar to those of *Haynaldia*. *Secale fragile*, with short auricle hairs, crossed with *Haynaldia* produced hybrids with auricle hairs as long as those of the *Haynaldia* parent.

16. The ligules of the various species and hybrids vary in length from 0.7 to 4.0 mm. *Triticum aegilopoides* and *T. timopheevi* crossed with *Haynaldia villosa* produced hybrids with ligules intermediate in length between those of the parents. *T. dicoccoides* and *T. turgidum* crossed with *Haynaldia* produced hybrids having ligules greater in length than those of either parent. *T. durum*, *T. polonicum*, and *Secale fragile* crossed with *Haynaldia* produced hybrids having ligules of the same length as those of the *Haynaldia* parent. The ligules of the F_1 hybrid of the cross *T. dicoccum* \times *Haynaldia* are similar in length to those of the *T. dicoccum* parent.

17. The spike widths of the F_1 hybrids are not exactly comparable with those of the parents because of self-sterility. When kernels are present in the florets the glumes are spread apart, causing an increase in the width of the spikelets. The simple type of head of *Triticum turgidum* var. *Alaska* is 10 mm wide, while the ramified type of the same variety is 35 mm wide. Hybrids of *T. turgidum* \times *Haynaldia villosa* produced simple heads with a maximum width of 9 mm or ramified heads with a maximum head width of 35 mm. The F_1 simple head type is about the same width as that of *Haynaldia*, the smaller parent, and the F_1 ramified heads are about the same width as those of the *T. turgidum* parents. In the crosses *T. aegilopoides* \times *H. villosa*, *T. dicoccoides* \times *H. villosa*, *T. durum* \times *H. villosa*, and *Secale fragile* \times *H. villosa* the F_1 heads are somewhat intermediate in width between those of the parents. The head width of the F_1 of *T. dicoccum* \times *H. villosa* is the same as that of *T. dicoccum*, the smaller parent, and the F_1 head width of the crosses *T. timopheevi* and *T. polonicum* with *H. villosa* is less than that of either parent (pls. 5, 9, 10, 11).

18. Crosses of the various species of *Triticum* and *Secale* with *Haynaldia villosa* produced F_1 hybrids with spikes longer than those of either parent (figs. 1-8).

19. The number of spikelets to a head varies with the parents and hybrids. *Triticum dicoccoides*, with an average of 19 spikelets, represents the species

with the smallest number of spikelets to the head. *T. turgidum*, with an average of 55 spikelets, represents the species with the greatest number of spikelets to the head. When *T. aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. polonicum*, and *Secale fragile* were crossed with *Haynaldia villosa* the F_1 hybrids produced a greater average number of spikelets to the head than that produced by either parent. When *T. dicoccum*, *T. durum*, and *T. turgidum* were crossed with *Haynaldia* the F_1 hybrids produced an average number of spikelets intermediate between those of the parents.

20. The spikelets vary in width. The width of the F_1 spikelets resulting from the crosses *Triticum aegilopoides*, *T. dicoccoides*, *T. turgidum*, and *Secale fragile* with *Haynaldia villosa* is greater than that of either parent. The width of the F_1 spikelets resulting from the crosses of *T. timopheevi* and *T. dicoccum* with *Haynaldia* is smaller than that of either parent. The cross of *T. durum* with *Haynaldia* produces a spikelet the width of which is intermediate between the widths of the parents. The width of the F_1 spikelets of the cross *T. polonicum* by *Haynaldia* is equal to that of *T. polonicum*, the larger parent (pls. 5, 9, 10, 11).

21. There is considerable variation in the length of the spikelets of the parents and hybrids. The spikelet measurements were made from the tip of the uppermost floret to the base of the glume of the lowermost floret. (Crosses of *Triticum aegilopoides*, *T. dicoccum*, and *T. polonicum* with *Haynaldia* produced F_1 hybrids with spikelets intermediate in length between those of the parents. *T. timopheevi*, *T. dicoccoides*, *T. turgidum*, and *Secale fragile*, when crossed with *Haynaldia*, produced F_1 hybrids greater in spikelet length than that of either parent. The F_1 hybrid of *T. durum* \times *H. villosa* produced spikelets equal in length to those of *T. durum*. Characters that materially influence the length of the spikelet are the number and length of the imperfect upper florets. In the species studied, with the exception of *T. aegilopoides*, each of the two lower florets of a spikelet normally produced a kernel. In all the hybrids but one (*T. turgidum* var. Alaska \times *H. villosa*) the glumes appeared normal in development but no kernels were produced. The number of imperfect upper florets of a spikelet varies somewhat among the different species. The upper florets of the spikelets of *S. fragile* and *T. aegilopoides* are only rudimentary and do not protrude above the two lower florets. *T. aegilopoides* produces only a single kernel to the spikelet. *Haynaldia* had the greatest number of sterile florets protruding on the spikelet and when crossed with species of *Triticum* and *Secale* usually produced an intermediate expression of this character (pls. 5, 9, 10, 11).

22. Branched rachis is dominant over simple rachis. *Haynaldia villosa* and all the species of *Triticum* studied, with the exception of *T. turgidum*, have simple rachises. *T. turgidum* var. Alaska usually produces ramified or branched heads, but this character is not stable under all environments and both branched and simple head types may be produced. When *T. turgidum* var. Alaska with ramified or branched heads was crossed with *H. villosa* having simple heads, the F_1 hybrids produced both branched and simple heads (fig. 7).

23. Fragile rachis is dominant over tough rachis. Fragility of the rachis has been divided into two types of expression, i. e., fragile and very fragile. *Triticum aegilopoides*, *T. dicoccoides*, *Secale fragile*, and *Haynaldia villosa* have very fragile rachises. *T. dicoccum* has a fragile rachis. *T. timopheevi*, *T. durum*, *T. polonicum*, and *T. turgidum* have tough rachises. Species with fragile rachises crossed with *H. villosa*, having a very fragile rachis, produced F_1 hybrids with very fragile rachises. Species with tough rachises crossed with *H. villosa* produced F_1 hybrids with very fragile rachises. The one exception to this was the F_1 hybrid of the cross *T. polonicum* \times *H. villosa*, which produced fragile rachises (pls. 5, 9, 10, 11).

24. All the species of *Triticum* and *Secale* used in crosses, with the exception of *T. timopheevi*, have oblong or wedge-shaped rachis internodes. When these were crossed with *H. villosa*, with obovate rachis internodes, the F_1 hybrids had intermediate-shaped internodes. *T. timopheevi*, with blunt cuneate-shaped rachis internodes, when crossed with *Haynaldia*, produced F_1 hybrids with rachis internodes intermediate between those of the parents. In plate 8, A, is shown an oblong rachis internode of *T. dicoccoides*; plates S, C, shows an obovate rachis internode of *H. villosa*; plate 8, B, the F_1 hybrid between the two forms. As may be observed, the F_1 hybrid favors the *Triticum* parent in length, while the obovate condition of the *Haynaldia* rachis predominates.

25. The rachis internodes of the various parents and F_1 hybrids vary in length. When *Triticum aegilopoides* and *T. durum* with long rachis internodes

were crossed with *Haynaldia villosa* with short rachis internodes, the internodes of the F_1 hybrids were the same length as the internodes of the *Triticum* parents. *T. timopheevi*, with the short rachis internodes, when crossed with *Haynaldia*, produced F_1 hybrids with internodes longer than those of either parent. *Secale fragile*, *T. dicoccoides*, and *T. polonicum*, with long internodes, when crossed with *Haynaldia* produced F_1 hybrids with internodes intermediate between those of the parents. The F_1 hybrids of the crosses *T. turgidum* and *T. dicoccum* with *Haynaldia* produced rachis internodes greater in length than those of the *Triticum* parent.

26. The nontufted condition of hairs on the lateral edges of the rachis internodes is dominant over the tufted condition. All of the species of *Triticum* and *Secale* studied have nontufted hairs on the lateral edges of the rachis internodes, and, while *Haynaldia* has rachis internodes with tufted hairs, the F_1 hybrids all had nontufted hairs (pls. 5, 9, 10, 11).

27. Glume width varies among the different species and hybrids. The glumes of the F_1 hybrids of *Triticum timopheevi*, *T. aegilopoides*, and *Secale fragile* with *H. villosa* are intermediate in width between the glumes of the parents. F_1 hybrids of *T. durum* and *Haynaldia* have glumes equal in width to those of *Haynaldia*, the parent with widest glumes. The F_1 hybrids of *T. polonicum* and *Haynaldia* are equal to *T. polonicum* in width of glume. The glume width of the F_1 hybrids of *T. turgidum* and *T. dicoccum* with *Haynaldia* is greater than that of either parent. The glumes of *T. dicoccoides*, *H. villosa*, and their hybrids are alike in width.

28. Glume length varies among the species and hybrids. Crosses of *Triticum timopheevi*, *T. dicoccoides*, *T. polonicum*, and *Secale fragile* with *Haynaldia* produced hybrids the glume length of which was intermediate between that of the parents. When *T. aegilopoides*, *T. dicoccum*, *T. durum*, and *T. turgidum* are crossed with *Haynaldia*, the F_1 glume length is greater than that of either parent.

29. The beaks or awns on the glumes of the several species of *Triticum* and of *Secale* and *Haynaldia* and their hybrids vary considerably in length. All of the *Triticum* species studied have shorter glume beaks than those of *Haynaldia*. The glume beaks of the F_1 hybrids of *T. timopheevi*, *T. dicoccoides*, and *T. dicoccum* are greater in length than those of the parents. F_1 hybrids of *T. aegilopoides*, *T. durum*, and *T. turgidum* have glume beaks intermediate between those of the parents but favor *Haynaldia*. The F_1 hybrid of *T. polonicum* and *Haynaldia* has glume beaks as long as those of *Haynaldia*, the parent with the longer glume beaks. The glume beaks of the F_1 hybrid of *S. fragile* and *Haynaldia* are intermediate between those of the parents but favor those of *Secale*.

30. Considerable variation occurs in the shape of the glumes at the apex and in the expression of the tooth when present. *Haynaldia villosa* has broad, elevated, and rounded shoulders. *Secale fragile* lacks glume shoulders, and when crossed with *H. villosa* it produced F_1 hybrids with glume shoulders slightly elevated and somewhat intermediate in width. When *Triticum durum*, with narrow and slightly elevated glume shoulders, was crossed with *H. villosa*, the F_1 hybrids had shoulders that were elevated and intermediate in width. *T. turgidum*, with narrow and oblique glume shoulders, crossed with *H. villosa*, produced F_1 hybrids having elevated glume shoulders intermediate in width. *T. monococcum* and *T. dicoccum*, with narrow glume shoulders and a short tooth, crossed with *Haynaldia*, produced F_1 hybrids having glume shoulders intermediate in width and possessing a tooth on each glume. *T. dicoccoides* and *T. timopheevi*, with glume shoulders slightly elevated and with a single tooth, when crossed with *Haynaldia*, produced F_1 hybrids with usually slightly elevated glume shoulders intermediate in width and sometimes showing a rudimentary tooth (pls. 5, 9, 10, 11).

31. *Haynaldia villosa* has large papillae on its glumes. When crossed with *Triticum aegilopoides*, *T. dicoccoides*, *T. durum*, *T. dicoccum*, *T. turgidum*, *T. polonicum*, and *Secale fragile*, all with small papillae, it produced F_1 hybrids with intermediate-sized papillae on their glumes. *T. timopheevi*, with glumes devoid of papillae, when crossed with *Haynaldia* produced F_1 hybrids with medium-sized papillae on their glumes.

32. Canaliculate glumes are dominant over the noncanaliculate condition. *Haynaldia villosa* possesses a deep channel or depression between the two prominent keels of the glume. No channels of this nature are present in the other species studied. The crosses all showed the canaliculate condition in the F_1 hybrids, except that the glumes had more shallow depressions than *H.*

villosa and were usually bisected by a prominent nerve, forming two distinct channels. *Secale fragile* \times *H. villosa* had no middle nerve. Unicanaliculation is always associated with bicarination.

83. Bicarinate glumes are dominant over unicarinate glumes. All of the species of *Triticum* and *Secale* reported herein are single-keeled or unicarinate, while *Haynaldia villosa* has bicarinate glumes. There is some variation in the keel expression of the bicarinate glumes of the F_1 hybrids, but, in general, the bicarinate condition predominates.

34. Glaucous glumes are dominant over nonglaucous glumes. *Haynaldia villosa*, *Triticum timopheevi*, *T. polonicum*, *T. turgidum*, *T. durum*, and *Secale fragile* have glaucous glumes, and hybrids involving them all had glaucous glumes. *T. dicoccum*, *T. aegilopoides*, and *T. dicoccoides* have nonglaucous glumes, yet when crossed with *H. villosa* the hybrids had glaucous glumes. The hybrids usually were glaucous, intermediate in degree between the parents.

35. Pubescent glumes and lemmas are dominant over nonpubescent glumes and lemmas. All of the species studied, except *Triticum polonicum* and *T. timopheevi*, have nonpubescent glumes and lemmas. The two exceptions have pubescence on the exterior surface of the glumes. The presence of pubescence on the glumes and lemmas of these forms is accompanied by the additional development of asperites and hairs (pubescence) of greater thickness and length on the keels and nerves. This is true to an extent that hairs sometimes resemble small superasperites (pl. 9). When *Haynaldia*, with glabrous glumes and tufted bristles on its keels, is crossed with *Triticum timopheevi*, with pubescent glumes and asperites on its keels, the F_1 hybrids have glumes with considerably reduced pubescence both in length of hair and distribution, and keels with superasperites 1.7 mm long. On the other hand, when *Haynaldia* is crossed with *T. polonicum*, with less pubescence than that possessed by *T. timopheevi*, the F_1 hybrids have a reduced pubescence on the glumes with only slight development of asperites on the keels and prominent nerves. These asperites rarely exceed 0.5 mm in length. A few superasperites as long as 1.5 mm, however, were sometimes developed on the keel of the lemma.

36. The lemmas of the different species and F_1 hybrids are variable in width. When *Haynaldia villosa* was crossed with *Triticum aegilopoides*, the F_1 hybrids had lemmas equal in width to those of *Haynaldia*, which had the widest lemmas. The F_1 hybrids of *T. dicoccoides* have lemmas equal in width to those of the parents. The F_1 lemma widths of the crosses *T. durum* and *T. turgidum* with *Haynaldia* are greater than those of either of the parents. The F_1 hybrids of the cross *Secale fragile* with *Haynaldia* have lemmas equal in width to those of *S. fragile*, the parent having the wider lemmas. The F_1 hybrids of the cross *T. dicoccum* \times *H. villosa* have lemmas as wide as those of *T. dicoccum*, the wider parent.

37. The lemmas of all the species and hybrids vary in length. In all the crosses but one the F_1 hybrids produced lemmas intermediate in width between those of the parents. The exception, the cross *Triticum dicoccum* \times *H. villosa*, produced F_1 hybrids with lemmas slightly greater in length than those of either parent.

38. The awns on the lemmas of the various species and their hybrids show considerable variation in length. Crosses of *Triticum aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. durum*, *T. polonicum*, *T. turgidum*, *T. dicoccum*, and *Secale fragile* with *Haynaldia villosa* produced F_1 hybrids with lemma awns intermediate in length between those of the parents.

39. Black or purple awns on lemmas and glumes are dominant over yellow awns. When black-awned species are crossed with purple-awned species, the awns of the hybrids are usually purplish black. *Triticum timopheevi*, *T. dicoccoides*, *T. polonicum*, and *T. turgidum* have brown to black awns, while *T. aegilopoides*, *T. dicoccum*, and *T. durum* have yellow awns. When *Haynaldia villosa* with purple pigment in its awns was crossed with *T. aegilopoides*, *T. dicoccum*, or *T. durum*, the F_1 hybrids produced weakly purple awns. When *H. villosa* was crossed on *T. timopheevi*, *T. dicoccoides*, *T. polonicum*, or *T. turgidum*, the F_1 hybrids had purplish black awns. *Haynaldia* crossed with *Secale fragile*, also having purple awns, produced F_1 hybrids with awns usually more intensely purple than those of either parent.

40. *Haynaldia villosa* has large papillae on its lemmas. When crossed with *Triticum aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. durum*, *T. dicoccum*, *T. turgidum*, *T. polonicum*, and *Secale fragile*, all with small papillae, F_1 hybrids were produced with intermediate-sized papillae on their lemmas. In plates 2, D, and 11 F. are shown papillae on a lemma of *H. villosa*.

41. In general, the trichomes on the glume keels of the F_1 hybrids resulting from crosses between species having short and long glume keel trichomes are intermediate in length. When *Triticum* species possessing glumes with asperites on their keels were crossed with *Haynaldia*, which possesses bristles on its glume keels, the F_1 hybrids had superasperites on the keels (pls. 5, 9, 10, 11, 12). *Secale fragile*, with asperites on its glume keels, when crossed with *Haynaldia*, produced F_1 hybrids with superasperites on their glume keels slightly shorter than those on the *Triticum* hybrids (pls. 10, 14).

42. Nontufted or continuous trichomes on the glume keels are dominant over tufted trichomes. Nontufted trichomes are present on the glume keels of the species of *Triticum* and *Secale* studied. When they were crossed with *Haynaldia*, which has tufted trichomes on its glume keels, the F_1 hybrids had nontufted trichomes or superasperites on their glume keels. These superasperites were usually fewer, thicker, and shorter than the tuft bristles on the glume keels of *Haynaldia* (pls. 5, 9, 10, 11, 12, 14).

43. *Triticum aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. polonicum*, and *T. turgidum* have short trichomes or asperites on the keels of their lemmas. When crossed with *Haynaldia villosa*, having long trichomes or bristles on the keels of its lemmas, these species produced F_1 hybrids with superasperites intermediate in length between the trichomes of the parents (pls. 5, 9, 10, 11, 12). *Secale fragile*, with spur asperites on its lemma keels, when crossed with *H. villosa* produced F_1 hybrids with superasperites intermediate between the trichomes of the parents (pls. 9, 14).

44. Nontufted or continuous trichomes on the lemma keels are dominant over tufted trichomes. Nontufted trichomes are present on the lemma keels of the species of *Triticum* and *Secale* studied. When these species were crossed with *Haynaldia*, which has tufted trichomes on its lemma keels, the F_1 hybrids had nontufted trichomes or superasperites on their lemma keels. These superasperites were usually fewer, thicker, and shorter than the tuft bristles on the lemma keels of *Haynaldia* (pls. 5, 9, 10, 11, 12, 14).

45. At the base and on the exterior side of the spikelets of the various species and hybrids are short bristles varying in length from 0.2 to 5 mm. *Triticum aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. polonicum*, and *T. turgidum* have basal bristles considerably longer than those of *Haynaldia villosa*. When these species were crossed with the latter, the F_1 hybrids had basal bristles intermediate in length between those of the parents. *Secale fragile*, with basal bristles 0.2 mm long, produced F_1 hybrids with basal bristles 0.35 mm long. Plate 6 shows the basal bristles of the parents and F_1 hybrid of the cross *T. dicoccoides* \times *H. villosa*.

46. In general, the number of bristles at the base and on the exterior side of the rachis of the F_1 hybrids is intermediate between those of the parents, but tends toward the condition in the *Triticum* or *Secale* parent (pls. 6, 7).

47. At the base of the spikelet on the side facing the rachis the various species and F_1 hybrids possess short bristles varying in length from 0.2 to 1.5 mm. *Triticum aegilopoides*, *T. dicoccum*, and *T. turgidum*, with bristles 0.3 mm long, produced F_1 hybrids with bristles equal in length to those of the *Triticum* parent. *T. durum*, *T. polonicum*, and *Secale fragile*, with basal bristles equal to or longer than those of *Haynaldia*, when crossed with the latter gave F_1 hybrids with bristles longer than those of either parent. *T. timopheevi* and *T. dicoccoides*, with basal bristles much longer than those of *Haynaldia*, when crossed with the latter gave F_1 hybrids with basal bristles intermediate in length between those of the parents. Plate 7 shows the basal bristles of the parents and F_1 hybrid of the cross *T. dicoccoides* \times *H. villosa*.

48. In general, the number of bristles at the base on the side of the spikelets facing the rachis of the F_1 hybrids is intermediate between those of the parents but tends toward the condition in the *Triticum* or *Secale* parent (pls. 6, 7).

49. The F_1 hybrids of the crosses *Triticum aegilopoides*, *T. timopheevi*, and *Secale fragile* with *Haynaldia villosa* had lodicules intermediate in width between those of the parents. F_1 hybrids of the crosses *T. dicoccum* and *T. durum* with *H. villosa* had lodicules greater in width than those of the parents. The cross *T. polonicum* \times *H. villosa* produced F_1 hybrids with lodicules as wide as those of the *T. polonicum* parent. In the cross *T. turgidum* \times *H. villosa* the F_1 lodicule width was as great as that of the *Haynaldia* parent. The F_1 hybrids of the cross *T. dicoccoides* \times *H. villosa* had lodicules similar in width to those of the parent (pl. 13).

50. The length of the lodicules of the various species of *Triticum*, *Secale fragile*, *Haynaldia villosa*, and their hybrids varies. Hybrids of the crosses

T. aegilopoides, *T. timopheevi*, *T. dicoccoides*, *T. durum*, and *T. turgidum* with *Haynaldia villosa* produced lodicules intermediate in length between those of the parents. F_1 hybrids of *T. polonicum* \times *H. villosa* produced lodicules longer than those of the parents. F_1 hybrids of *T. dicoccum* \times *H. villosa* had lodicules as long as those of *Haynaldia*, the longer parent. The F_1 hybrids of *S. fragile* \times *H. villosa* had lodicules as long as those of the *Secale* or longer parent (pl. 13).

51. The length of the hairs on the lodicules is variable. Short hairs are present on both the exterior surface and edges of the lodicules of the *Triticum* species. *Secale fragile* has long hairs confined mostly to the edges of the lodicules, and *Haynaldia villosa* has lodicules with only a few very short hairs on the edges or none at all. The species of *Triticum* and *Secale* used in the crosses had hairs with a maximum length of 1.35 mm on their lodicules, while *H. villosa* had lodicule hairs with a maximum length of 0.05 mm. With the exception of the F_1 hybrids from the crosses *T. aegilopoides* and *T. dicoccoides* with *H. villosa*, all the crosses produced F_1 hybrids with lodicule hairs intermediate in length between those of the parents. The two exceptions produced F_1 hybrids with lodicule hairs longer than those of the *Triticum* or longer parent (pl. 13).

52. *Triticum aegilopoides* has paleas that are split laterally through the center. All of the other species of *Triticum*, *Secale fragile*, and *Haynaldia villosa* have entire paleas. When *T. aegilopoides*, with split paleas, was crossed with *H. villosa*, the F_1 hybrids produced entire paleas.

The only F_1 hybrid involved in the experiments herein reported that produced seed was that from the cross *Triticum turgidum* var. Alaska \times *Haynaldia villosa*. The kernels of this F_1 hybrid are longer than those of either parent and somewhat intermediate in width. The kernels of the F_2 plants are similar in size to those of the F_1 plants. Plate 2 shows characteristic kernels of the parents and of the F_1 and F_2 .

With the exception of the cross *Triticum turgidum* var. Alaska \times *Haynaldia villosa*, no material is available for study beyond the F_1 generation, owing to self-sterility. However, the variability in the expression of the characters of the parents and their behavior in the F_1 hybrids suggests different modes of inheritance. The following characters show a quantitative expression: Plant stature, leaf width, auricle length, auricle hair length, ligule length, width and length of spike, number of spikelets, width and length of spikelets, length of rachis internodes, length and width of glumes, length of glume beak, length and width of lemma, awn length, length of trichomes on glume and lemma keels, length of bristles at base of spikelet on exterior side and on side facing rachis, width and length of lodicule, and length of lodicule hairs.

The characters that are extremely difficult or impossible to measure at the present time, but which, by inspection, also showed differences in expression of quantitative nature are as follows: Solidity of straw, anthocyanin in culm and leaf sheath, glaucousness of culm and leaf sheaths, pubescence of culm nodes and glumes, scabrousness of leaf-blade margins, pubescence of leaves and leaf sheaths, glaucousness of glumes, fragility of rachis, shape of shoulder on glume, canalicularity of glumes, carination of glumes, black and purple pigmentation of the awns, and number of bristles at the base of the spikelet on exterior and interior sides.

Other characters that suggest simple inheritance based on single-factor differences are pubescence of the peduncle, pubescence on the overlapping edge of leaf sheaths, branching of rachis, size of papillae on lemma and glume, arrangement of trichomes (tufting) on glume and lemma keels, and split palea.

SUMMARY

Haynaldia villosa was crossed on *Triticum aegilopoides*, *T. timopheevi*, *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. polonicum*, *T. turgidum*, and *Secale fragile*, and the F_1 plants were matured. Attempts also were made to cross *H. villosa* with *T. vulgare*, *T. compactum*, *T. spelta*, *S. cereale*, and *S. cereale ancestrale*, but only an occasional enlarged structure, with neither embryo nor endosperm, was produced. However, one hybrid seed of the cross *T. vulgare* (C. I. 6223) \times *H. villosa* germinated but the plant died before the formation of the third leaf.

All the F_1 plants except those of the cross *Triticum turgidum* var. Alaska \times *Haynaldia villosa* were completely self-sterile. The F_1 hybrids of this cross produced an average seed set of 3.8 percent, with a maximum of 11.1 percent for a single plant. The F_2 plants produced an average seed set of 29.7 percent, with a maximum of 58.8 percent for a single plant. The F_3 plants produced an average seed set of 58.5 percent, with a maximum of 76.9 percent for a single plant. No apparent segregation occurred in the F_2 and subsequent generations, the F_1 type remaining fixed in its morphological characters.

In general, most of the F_1 hybrids resulting from the crossing of *Triticum* species and *Secale fragile* with *Haynaldia villosa* resembled the *Triticum* or *Secale* parent, but a critical study of the morphological characters possessed by the parents and the F_1 hybrids indicates that the majority of the characters of the hybrids are intermediate between those of the parents. Some characters of the F_1 plants show, however, a decided increase in degree of expression over those of either parent, while others show a dominance of the one or the other parent.

More than 52 morphological characters of the parents and the F_1 hybrids of eight crosses were studied in detail.

LITERATURE CITED

- (1) BLEIER, H.
1928. GENETIK UND CYTOLOGIE TEILWEISE UND GANZ STERILER GETREIDE-BASTARDE. *Bibliog. Genetica* 4: [321]-400.
- (2) ———
1928. ZYTOLOGISCHER UNTERSUCHUNGEN AN SELTENEN GETREIDE UND RÜHEN-BASTARDEN. *Verhandl. 5th Internatl. Kong. Vererbungswissenschaft*, Berlin, v. 2, pp. [447]-452.
- (3) KAJANUS, B.
1927. DIE ERGEBNISSE DER GENETISCHEN WEIZENFORSCHUNG. *Bibliog. Genetica* 3: [141]-244.
- (4) LONGLEY, A. E., and SANDO, W. J.
1930. NUCLEAR DIVISIONS IN THE POLLEN MOTHER CELLS OF TRITICUM, AEGILOPS, AND SECALE AND THEIR HYBRIDS. *Jour. Agr. Research* 40: 683-719, illus.
- (5) OEHLER, E.
1933. UNTERSUCHUNGEN ÜBER ANSATZVERHÄLTNISSE, MORPHOLOGIE UND FERTILITÄT BEI AEGILOPS-WEIZENBASTARDEN. I. TEIL. DIE F_1 -GENERATION. *Ztschr. Induktive Abstam. u. Vererbungslehre* 64: [95]-153, illus.
- (6) RAINERI, L.
1914. LA STAZIONE DI GRANICOLTURA DI RIETI. *Italia Agr.* 51: 6-12.
- (7) TSCHERMAK, E.
1921. BEITRÄGE ZUR VERVOLLKOMMUNG DER TECHNIK DER BASTARDIERUNGSZÜCHTUNG DER VIER HAUPTGETREIDEARTEN. *Ztschr. Pflanzenzücht.* 8: 1-13, illus.

- (8) TSCHERMAK, E.
1929. EIN NEUER FRUCHTBARER WEIZENARTBASTARD (TRITICUM TURGIDUM \times TRITICUM VILLOSUM). In Forschungen auf dem Gebiete des Pflanzenbaus und der Pflanzenzüchtung. Festschrift für Kurt von Rümker. pp. [69]–80, illus. Berlin.
- (9) ———
1930. NEUE BEOBSACHTUNGEN AM FERTILEN ARTBASTARD TRITICUM TURGIDOVILLOSUM. Ber. Deut. Bot. Gesell. 48: 400–407.
- (10) TSCHERMAK-SEYSENEGG, E.
1933. WEITERE STUDIEN AM FERTILEN, KONSTANTEN ARTBASTARD TR. TURGIDOVILLOSUM UND SEINEN VERWANDTEN I. TEIL. Ztschr. Induktive Abstam. u. Vererbungslehre 66: 180–218, illus.
- (11) VERUSHKINE, S., and SHECHURDINE, A.
1933. HYBRIDS BETWEEN WHEAT AND COUCH GRASS . . . Jour. Heredity 24: 329–335, illus.
- (12) WATKINS, A. E.
1930. THE WHEAT SPECIES: A CRITIQUE. Jour. Genetics 23: [173]–263, illus.

A CYTOLOGICAL STUDY OF PUCCINIA MALVACEARUM FROM THE SPORIDIUM TO THE TELIOSPORE¹

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INTRODUCTION

As knowledge of heterothallism in the rusts broadens and the importance of the spermogonium in reproduction is realized, the question arises as to how reproduction takes place in rusts that have no spermogonia.

Hollyhock rust, *Puccinia malvacearum* Bert., a microcyclic species without spermogonia, was chosen for study. The rust is maintained by a succession of telial generations. Aecia, uredia, and spermogonia are unknown. An earlier paper (5)³ on this rust presents cytological details of the development of the teliospore, nuclear fusions within it, the germination of the spore, the reduction divisions, and the formation of the sporidia. The present study begins with the sporidium and traces the infection of the host, mycelial growth, and the development of the telial sorus.

The literature on *Puccinia malvacearum* was reviewed in the former paper (5). A few additional notes are given here.

Before the discovery of heterothallism in rusts (18), in 1927, homothallism was taken for granted. On the assumption that the binucleate mycelium could arise directly within an isolated haploid mycelium, the main cytological interest in both long- and short-cycle rusts centered on the mode of transition to the binucleate condition. Several papers deal with this point in *Puccinia malvacearum*.

Blackman and Fraser (15), in 1906, found that the transition from uninucleate to binucleate cells in *Puccinia malvacearum* took place at several points in the same sorus and suggested the possibility that two sister nuclei may become conjugate in a cell. They also state (15, p. 42) that —

The smallness of the cells and nuclei, and the absence of any regular row or group of cells—such as are found in the aecidia—on which attention can be concentrated in the hunt for nuclear migrations or cell-fusions, render the task of elucidating such a point almost hopeless.

Olive (29), in 1911, noted that in some short-cycle rusts the binucleate mycelium arises at the base of the sorus and that in others it “arises at some indefinite point earlier in the life history in the vegetative mycelium.” *Puccinia malvacearum* is listed in the first group.

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³ Reference is made by number (italic) to Literature Cited, p. 316.

Werth and Ludwigs (32), in 1912, found that in the young sorus of *Puccinia malvacearum* there is a definite layer of column-shape cells. These form pairs, each consisting of a smaller and a larger cell, and the nucleus of the smaller cell passes through a small opening in the walls into the larger cell. They also note an occasional irregular binucleate cell in the tissue above.

Moreau (28), in 1914, also found a layer of club-shape cells in the young sorus. These fuse by pairs, the wall between the two dissolving first at the tip, then through a broader contact area. The two cells that fuse may be either equal or unequal. The fusion cell gives rise to a short hypha of binucleate cells, at the tip of which the teliospore forms.

Lindfors (26), in 1924, also working on *Puccinia malvacearum*, saw fusions at an earlier stage, before the pseudoparenchyma of the sorus formed, and found that the cells that fuse may be equal or unequal. These earlier binucleate cells grow and divide further before spore formation.

As may be seen, these accounts of the origin of the binucleate cells in *Puccinia malvacearum* contradict each other on nearly every point.

Since the discovery of heterothallism in rusts, it still has been assumed that a microcyclic species without spermatogonia would be without means of crossing and so must be homothallic. Jackson (24), in 1931, and Buller (17, p. 286), in 1931, cite *Puccinia malvacearum* as probably homothallic, and Ashworth (12), in 1931, presents experiments in support of homothallism.

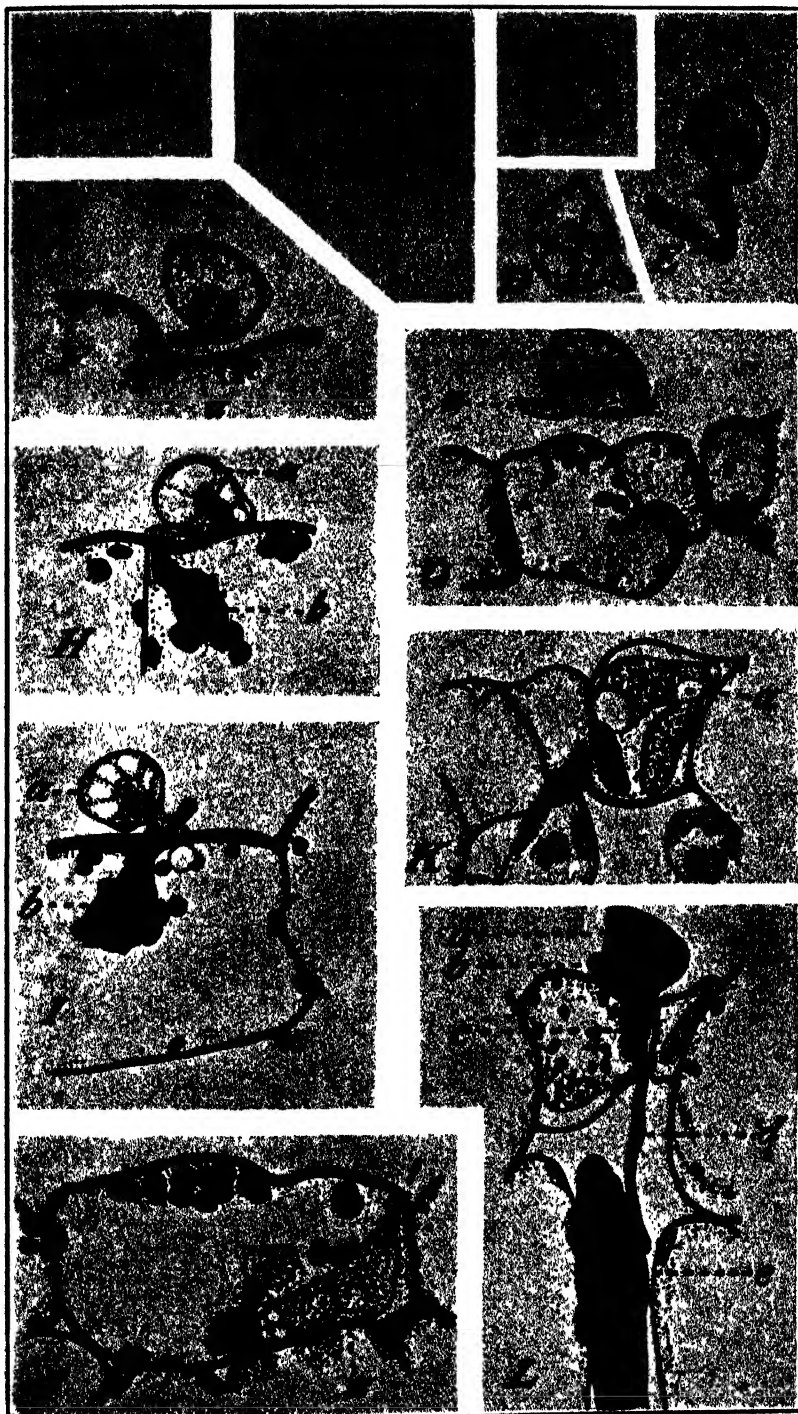
MATERIAL AND METHODS

Potted mallow (*Malva* sp.) plants were used for inoculation. Some were grown and inoculated in the greenhouse; others out of doors on a third-floor balcony. In inoculation, a glass cylinder 4 inches in diameter and 8 or 10 inches high, lined with wet paper toweling, was set down over the plant with the lower edge pressed into the soil. A pad of wet paper was placed in a Petri dish and an infected leaf of hollyhock (*Althaea rosea* (L.) Cav.) or mallow was placed on it and secured in position by rubber bands. This was inverted and placed on the top of the cylinder as a lid. A layer of wet paper was folded down about the top, covered with cellophane, and fastened in place by a rubber band. This placed the germinating spores directly over the mallow plant in a small damp chamber. The plant was then heavily watered and placed in diffuse light for 48 hours; then the cylinder was removed and the plant was placed in stronger light. Attempts were made to keep the plants free of insects.

Material was fixed daily up to 12 days after inoculation and at longer intervals from then until 25 days after inoculation. Several fixing solutions were tried, but Flemming's medium and weak solu-

EXPLANATORY LEGEND FOR PLATE 1

- A, B, C.—Mature binucleate sporidia 1 day after inoculation was set up. $\times 1,400$.
 D, E.—Germinating sporidia, 1 day after inoculation was set up. $\times 1,400$.
 F, G.—Sporidia on leaf, each with germ tube, a, 1 day after inoculation was set up. $\times 1,400$.
 H.—Cytoplasm from sporidium, a, flowing into host cell at b; 1-day infection. $\times 1,400$.
 I.—Germinating sporidium, a, at c. Beginning of primary hypha at b. One-day infection. $\times 1,400$.
 J.—Unicellular, binucleate primary hypha, a, from 1-day infection. $\times 1,400$.
 K.—Young primary hypha, a, in guard cell of stoma; 1-day infection. $\times 1,400$.
 L.—Sporidium, b, has entered guard cell at c. Sporidium, a, has entered stomatal aperture. Sporidium, a, germ tube, d, and palisade cell, e, are dead. Three-day infection. $\times 1,400$.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

tions proved the best. The fixed material was washed, dehydrated, and embedded in paraffin. The principal stain used was iron haematoxylin counterstained with Congo red.

In the search for conidia, both potted material and separate leaves of mallow and hollyhock were placed in damp chambers. Material was removed at different times during the day and night; part of it was fixed, and the rest was used in making freehand sections.

EXPERIMENTAL RESULTS

ENTRANCE AND VEGETATIVE GROWTH

During the first 24 hours after an inoculation is set up, the teliospores germinate and produce the sporidia, which fall upon the leaves of the host plant, germinate there, and enter the epidermal cells of the leaf.

The single nucleus of the immature sporidium of *Puccinia malvacearum* divides once (5) as the spore matures. Sporidia that have been discharged from the promycelium and are found lying on the host leaf are regularly binucleate (pl. 1, A, B, C).⁴ The spore is originally lemon-shape (C) but quickly flattens down against the epidermis (A, B) and pushes out a germ tube at one side (D; F, a; G, a). This germ tube usually is short, but occasionally (E; L, d) exceeds the diameter of the spore in length.

The tip of the germ tube penetrates the outer wall of the epidermal cell directly (pl. 1, H) and enters the host cell. In H the cytoplasm is flowing through the germ tube into the host cell (H, b), leaving the sporidium (a) somewhat vacuolated. In I, there are 2 spores, 1 of which (a) has germinated but not entered, while the other (with spore wall missing) has formed a sack-shape mass (b) inside the epidermal cell. Then the two sporidial nuclei move in (J, a), completing the transfer of the sporidial protoplast to the epidermal cell. The empty, collapsed sporidial wall (pl. 2, A, a; B, a; C, a; D, a) is often found at the point of entrance.

Ordinarily, the sporidium germ tube enters the epidermal cell on which the sporidium lies. This small thin-walled, short-lived cell with watery content and the minimum of food is incapable of producing a germ tube long enough to grow to a stoma. Even when, by chance, the sporidium falls upon a stoma, it may enter the guard cell instead of growing in through the stomatal aperture. In plate 1, K, which shows a diagonal section through a stoma, a portion of a badly cramped primary hypha is seen in the little guard cell at a. Perhaps when the sporidium landed the stoma was closed. Rarely, the germinating sporidium does enter the stomatal aperture. L shows two

⁴ With the exception of plate 5, A and B, drawings are oriented in the plates as the tissues drawn were oriented in the leaf, i. e., the part drawn that was nearest the upper epidermis of the leaf becomes the upper edge of the drawing.

EXPLANATORY LEGEND FOR PLATE 2

A.—At a, empty sporidial wall; b, enucleate and, c, binucleate cells of primary hypha of 1-day infection. $\times 1,400$.

B.—Primary hypha with empty sporidial wall at a, and septum at b dividing former binucleate cell into two uninucleate cells which have produced the branches, c, d; 2-day infection. $\times 1,400$.

C.—Four-cell primary hypha with sporidial wall at a, and branches in palisade cells at b and c; 2-day infection. $\times 1,400$.

D.—Primary hypha, b, c, with sporidial wall at a, and branches in palisade cells at d and e; 3-day infection. $\times 1,400$.

E.—Older primary hypha from 5-day infection. $\times 1,400$.

sporidia that fell upon the same stoma; the germ tube of one (*b*) entered the guard cell at *c*, while that of the other (*a*) grew in through the stomatal aperture. The venture was not a success. The stoma closed, pinching the germ tube. The sporidium (*a*), the germ tube (*d*), and even the palisade cell (*e*), which was in contact with the germ tube, have died.

The fungus newly entered in an epidermal cell (pl. 1, *J*, *a*) is unicellular and binucleate. It grows rapidly into a multicellular primary hypha. The first septum (pl. 2, *A*) divides the fungus into two nearly equal cells, the one nearest the point of entry (*b*) being without a nucleus, the other (*c*) being binucleate.

During the second day after inoculation, the primary hypha grows apically, becoming curled up in the epidermal cell. A second septum (pl. 2, *B*, *b*) soon cuts the binucleate cell into uninucleate cells, the terminal one of which continues the growth and divides again. *C* shows a 4-cell primary hypha. Early in this development the primary hypha branches. In *B* branches from the second and third cells (*c*, *d*) have grown down to the inner epidermal wall, and in *C*, *b*, *c*, the two branches have grown on into palisade cells, each taking with it the nucleus of the cell from which it grew. In *D* (from 3-day material) the primary hypha (*b*, *c*) has given rise to at least 4 branches, 2 of which have formed large, 2-cell hyphae (*d*, *e*) in palisade cells. Here the nuclear behavior seems to have been different from that shown in *C*, for each cell of the primary hypha still possesses a nucleus, except, of course, the first cell (*D*, *b*), which is now much shrunken.

When the epidermal cell containing a primary hypha is large, the primary hypha may continue to grow. In plate 2, *E* (from a 5-day infection), the richly branched primary hypha is made up of 7 or 8 cells. Sooner or later, however, the primary hypha deteriorates. It can rarely be identified with certainty after the ninth day.

Contrary to what is usually seen in rusts, the mycelium of *Puccinia malvacearum* is not strictly intercellular. Hyphae grow into and through the host cells with comparative freedom. In plate 3, *A*, a branch from a primary hypha entered a palisade cell at *a*, formed there a branching hypha of eight cells, from which at *b* and *d* branches passed into other palisade cells, while at *c* a branch grew out into a large intercellular space and branched rapidly there. Intracellular mycelium is coarser than intercellular mycelium; its cells have about twice the diameter of the intercellular growth. Intracellular growth permeating several palisade cells is shown in *B*. One branch from a primary hypha at *a* formed a slender intercellular hypha (*a*, *d*), from which at *c* a branch grew through one palisade cell into a second at *b*. A dozen or more palisade cells may be invaded in this fashion by branches from a single primary hypha. In plate 3, *C*, *D*, and plate 4, *A*, are shown further examples of the coarse, branching, intracellular hyphae in palisade cells.

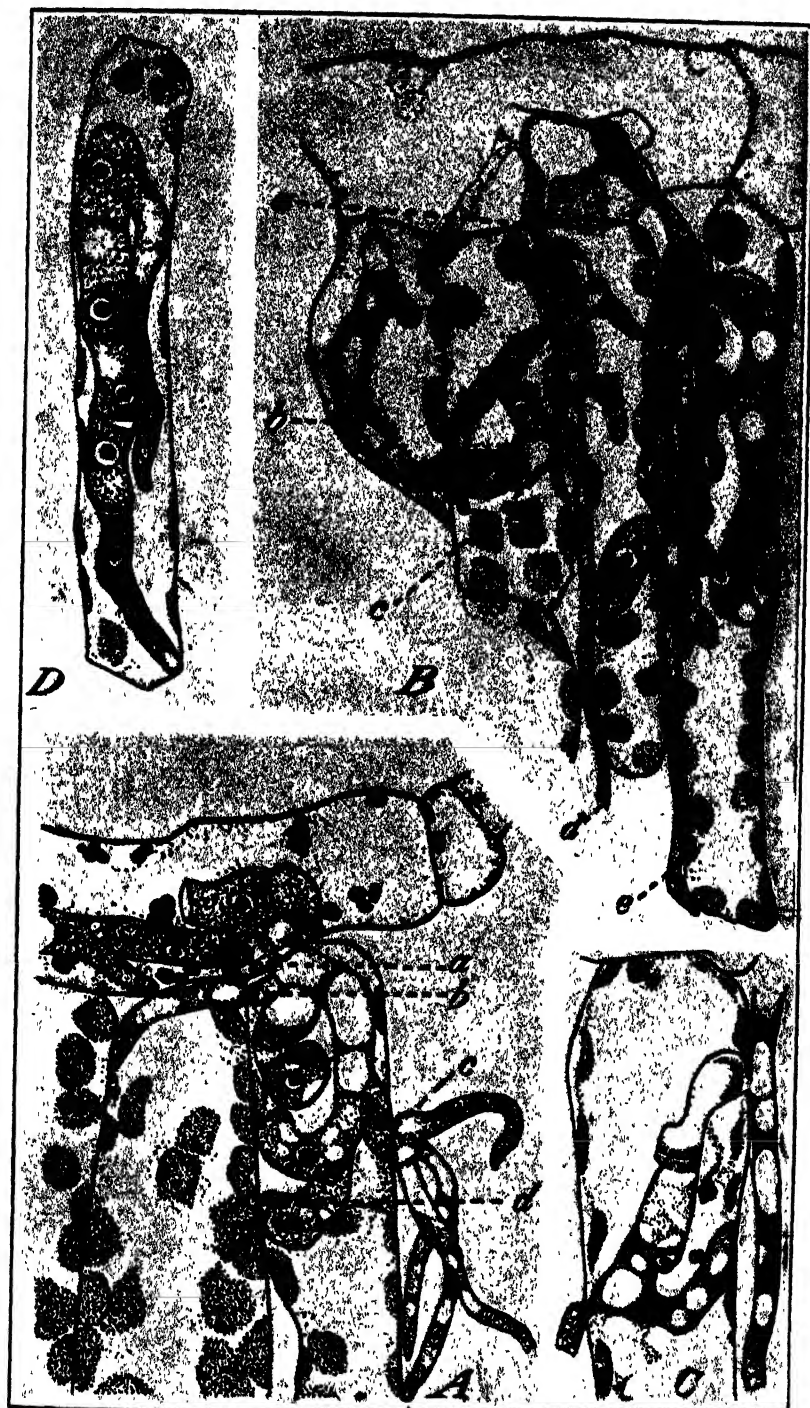
This intracellular habit is not limited to the early mycelial development. In plate 4, *B*, is shown a much-curved branching hypha of

EXPLANATORY LEGEND FOR PLATE 3

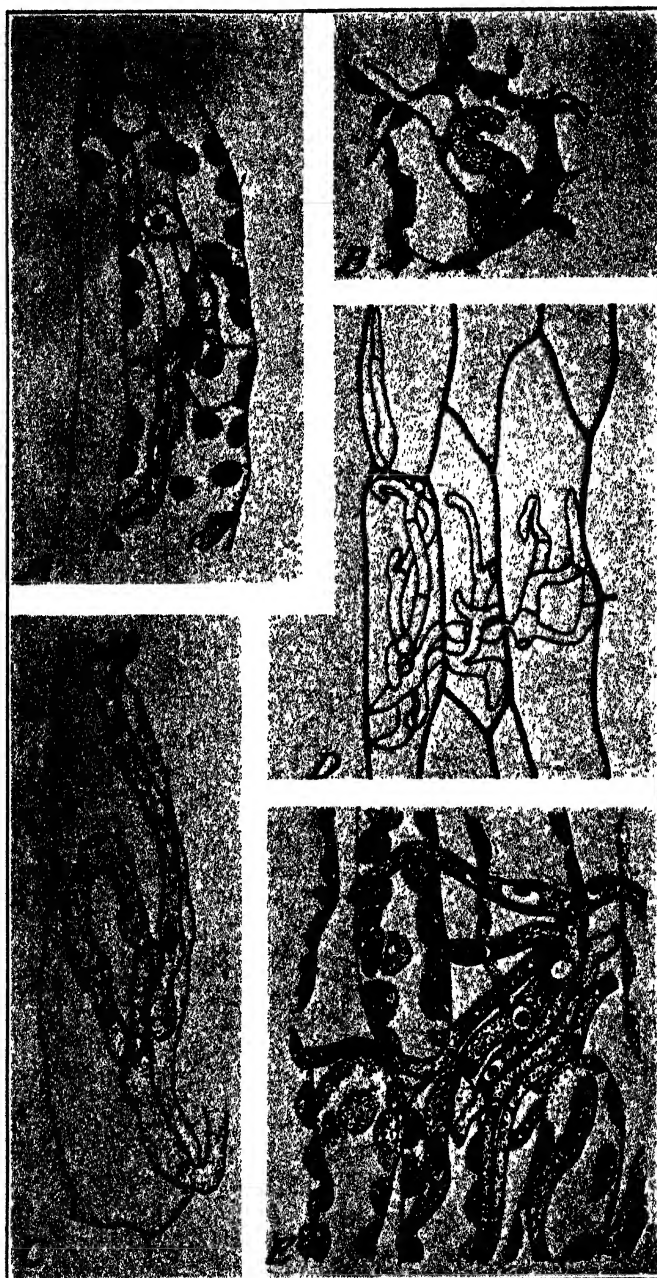
A.—A branch from a primary hypha at *a* gave rise to an intracellular hypha in a palisade cell with branches to other palisade cells at *b* and *d*, and a branch to intercellular spaces at *c*; from 5-day infection. $\times 1,400$.

B.—Intercellular mycelium at *a*, *d*, and at *e*, and intracellular hypha, *b*, *c*; 3-day infection. $\times 1,400$.

C, *D*.—Intracellular hyphae in palisade cells from 6-day infection. $\times 1,400$.



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5 or 6 cells in a spongy mesophyll cell, and in *C* a branching hypha in a large bundle-sheath cell. Similar hyphae often are found in the cells of the lower epidermis. These intracellular hyphae doubtless serve as haustoria in extracting food for the fungus, but they are more than haustoria, for they frequently grow through a host cell and out into intercellular spaces again. Their effect upon the host cell, too, is more severe than that of ordinary haustoria; invaded cells may die sooner.

Some intercellular growth is found between palisade cells near the point of entrance (pl. 3, *A, c; B, d, e*). From here the hyphae spread downward. Plate 4, *E*, shows a richly branching group of intercellular hyphae radiating out into the mesophyll area and entering freely the host cells encountered on the way.

When a sporidium by chance lands on a leaf over a large vein, it enters and develops there. Prevented by the vein from direct downward growth, the mycelium spreads in the tissue between the vein and the upper surface. Such infections show exceptional intracellular growth in the upper epidermis. Plate 4, *D*, represents a bit of such epidermal mycelium drawn from a freehand tangential section of living material.

So far as noted, this mycelium, whether intercellular or intracellular, is haploid in character. A terminal cell about to divide may be momentarily binucleate, but in general the cells are uninucleate.

NUCLEAR DIVISION AND NUCLEAR LOCOMOTION

As a hypha grows apically, the terminal cell elongates, its nucleus divides, and a septum divides this binucleate cell into two uninucleate cells. Division figures of vegetative nuclei are minute. The little that has been learned about mitosis is recorded here:

Plate 5, *A, a*, shows a resting nucleus. It is spherical or ellipsoidal and contains a large central spherical body. It is not certain whether the latter corresponds to the nucleole of higher plants or whether it includes chromatin. In addition, a delicately stained network can sometimes be seen spreading through the nuclear cavity. In *b* is shown what may be a prophase of division. As no detail can be made out in the irregular, dark-stained little mass, no certain identification of the stage is possible. In *c*, is shown what probably is an equatorial plate. The figure is too small to permit a count of the chromosomes. In reproductive areas of this rust (*5*), where division figures are larger, a count shows that the number is ± 5 . In *d* is shown an anaphase. Part of the chromosomes have divided and passed to the poles; the rest are still near the equator. In *e*, the chromatin is all at the poles and the two daughter nuclei are connected by a slender strand, which is perhaps the "central spindle." In *f* and *g* the two small daughter nuclei move toward each other, perhaps drawn together by the contraction of the connecting strand. No details of septum formation between the two nuclei have been seen.

EXPLANATORY LEGEND FOR PLATE 4

- A*.—Intracellular hypha in palisade cell from 6-day infection. $\times 1,400$.
- B*.—Intracellular hypha in spongy mesophyll cell; 6-day infection. $\times 1,400$.
- C*.—Intracellular hypha in a bundle-sheath cell; 6-day infection. $\times 1,400$.
- D*.—Surface view of intracellular mycelium in upper epidermis from 7-day infection located over a vein $\times 400$.
- E*.—Intercellular mycelium from 6-day infection. $\times 1,400$.

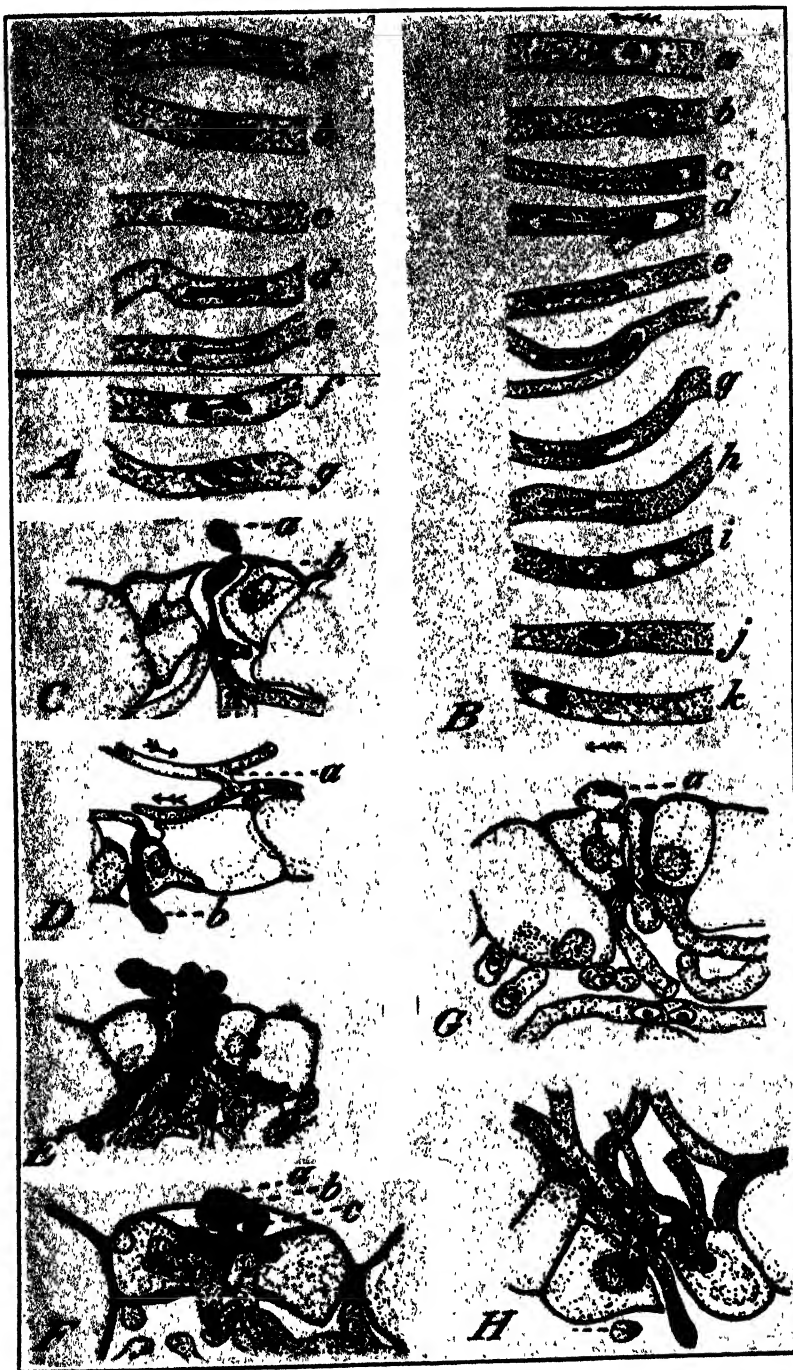
As soon as division is completed, each of the daughter nuclei moves toward the center of its own cell. When a hypha branches, a nucleus moves out into the branch. When an intracellular hypha forms, a nucleus moves into the hypha through the narrow opening in the host cell wall. An older theory assumed that, in this translocation of fungus nuclei incidental to vegetative growth, the streaming of the cytoplasm carried the nuclei along passively. A study of nuclei in actively growing areas throws doubt on this assumption. Moreover, during diploidization fungus nuclei can migrate through hyphae for considerable distances. By no stretch of the imagination can this be thought of as brought about by cytoplasmic streaming. It must be conceded that nuclei possess some form of locomotion.

Nuclear locomotion has been studied in the actively growing tips of hyphae of young haploid vegetative mycelium. Even a casual survey shows that nuclei here vary greatly in shape. Drawings were made representing the full range of variation. A study of these drawings shows that they can be arranged in a sequence that is at least suggestive of the mode of locomotion of the fungus nucleus. In order to make a beginning at understanding nuclear locomotion, the following tentative interpretation is offered.

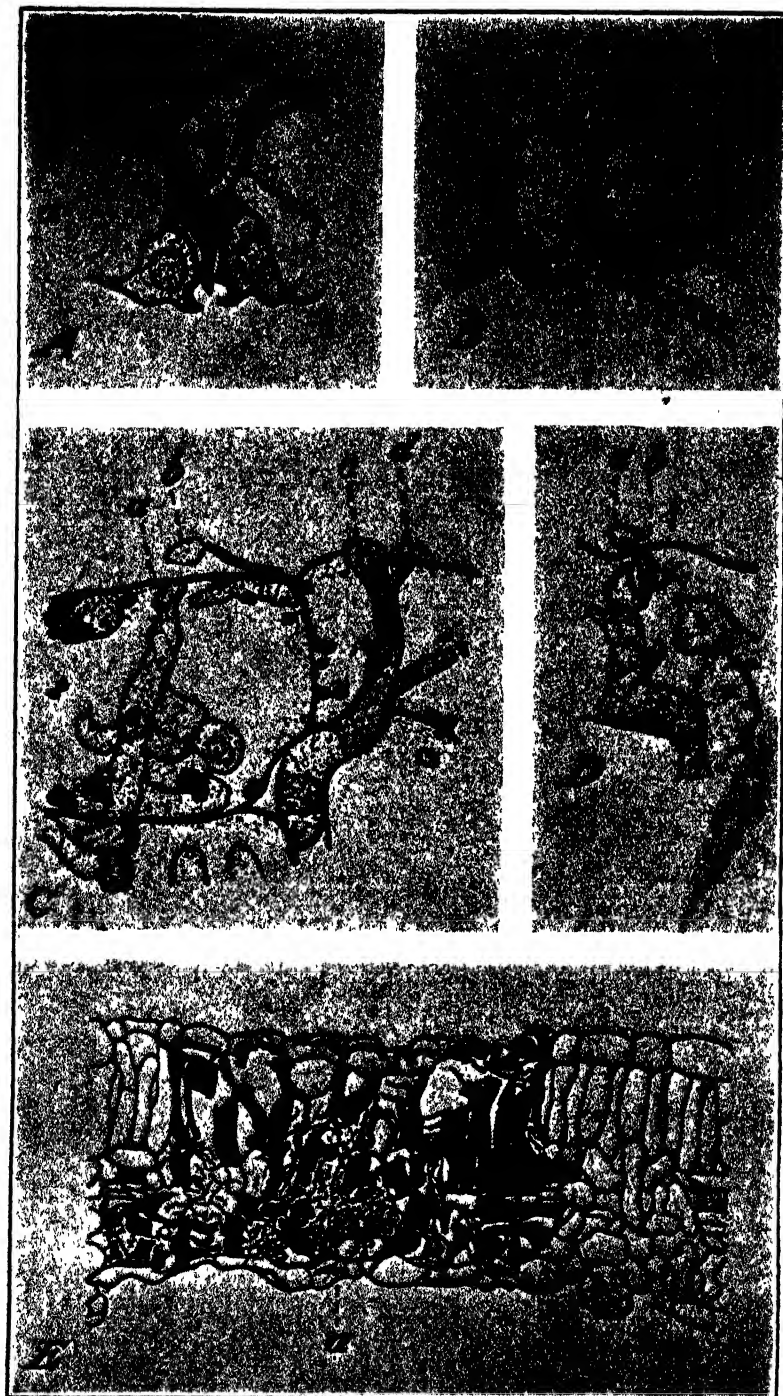
Plate 5, *B*, *a* to *k*, shows a representative series of nuclear shapes. The arrows indicate the direction of growth of the hypha, i. e., the growing tip of each hypha is at the left of the part drawn. A nucleus in repose (*a*) is spherical or ellipsoidal and is surrounded by undisturbed cytoplasm. Conspicuous within the nucleus is a central spherical body, which, as noted earlier, may be just a nucleole or may include chromatin. In *b* the nucleole has pushed out a slender rod in the direction of the apex of the hypha. This rod stretches the nuclear membrane into a short beak. In *c*, the beak has elongated to a fine tapering hair several times as long as the body of the nucleus. The length of this beak determines the length of the forward stride of the nucleus. In *d* and *e*, material appears to be flowing out from the body of the nucleus into the fine beak. At this stage, the beak is apt to be uneven in diameter, slender in some parts and bulging in others. At *f*, the point of origin of a branch hypha, a nucleus evidently has divided and a septum has formed between the two daughter nuclei. The distal daughter nucleus is now moving on toward the apex of the parent hypha, while the proximal daughter nucleus is moving out into the branch. In *g* and *h*, the evacuation of the old nuclear position is completed, the space being occupied by a rapidly collapsing vacuole, while the elongated rod of nuclear material is contracting into a more compact mass in the new location. It may be that *i* and *j* are later stages in this contraction, while *k* represents the reconstructed nucleus.

EXPLANATORY LEGEND FOR PLATE 5

- A.—Stages, *a* to *g*, in mitosis in vegetative hyphae from 6-day infection. × 2,200.
- B.—Nuclear shapes, *a* to *k*, found in rapidly growing vegetative hyphae, arranged in a sequence which suggests mode of nuclear locomotion; from 6-day infection. × 2,200.
- C.—Stomatal hypha, *a*, *b*, in upper epidermis of 7-day infection. × 1,400.
- D.—Detail of lower epidermis of 7-day infection with anastomosis, *a*, and stomatal hypha, *b*. × 1,020.
- E.—Hyphae with conidia in stoma of upper epidermis of 8-day infection. × 1,020.
- F.—Longitudinal section of stoma with detached conidium at *a*, and young conidia on stomatal hyphae at *b* and *c*; 8-day infection. × 1,400.
- G.—Hyphae in stoma of upper epidermis of 8-day infection with conidium at *a*. × 1,400.
- H.—Hyphae in stoma of lower epidermis with conidium at *a*; 8-day infection. × 1,400.



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If the nuclear forms shown in plate 5, *B*, *a*, *b*, and *c*, had been the only ones found, it would have been assumed that the long beak in *c* served as a whiplash and that the nucleus swam ahead by something akin to ciliary motion. Such a hypothesis, however, takes no account of the other nuclear forms.

These various forms of the nucleus are encountered frequently, the majority being represented in every section of an infection. A count of 100 nuclei in the growing tips of hyphae of a 6-day infection and a similar count of 100 nuclei in a 7-day infection gave the results shown in table 1. Nuclei in any stage of mitosis were excluded from the count.

TABLE 1.—Number of nuclei at each stage of nuclear locomotion among nuclei found in tips of hyphae 100 nuclei each from a 6-day and a 7-day infection of *Puccinia malvacearum*

Age of infection (days)	Number of nuclei at indicated stage ¹										Total	
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	<i>h</i>	<i>i</i>	<i>j</i>		<i>k</i>
6.....	44	7	4	9	4	1	5	8	3	11	4	100
7.....	53	7	3	10	8	2	3	7	1	5	1	100

¹ See pl. 5, *B*, *a* to *k*, for stages represented by letters.

As will be seen, even in rapidly growing hyphae, about half of the nuclei are in repose, while the rest present one or another of the appearances here interpreted as some stage of locomotion. The stage shown in plate 5, *B*, *c*, is probably more abundant than the tabulated numbers indicate. The long slender beak is easily overlooked and if faintly stained is invisible.

Early workers in rusts saw nuclear figures corresponding to those shown in plate 5, *B*, *d*, *e*, and *h*, and formulated on them the now obsolete theory of amitosis of vegetative fungus nuclei. It is a real question, however, whether any of these nuclear figures may be stages of mitosis. *A*, *b*, supposedly a prophase of division, and *B*, *j*, supposedly a stage of nuclear condensation after a forward stride, are markedly similar. There is much less risk of mistaking any other stage of mitosis for a stage of nuclear locomotion.

SURFACE HYPHAE

Early in the development of vegetative mycelia, hyphae grow to both the upper and the lower surface of the leaf. Hyphae grow into stomatal apertures, grow out through epidermal cells and, more rarely, force a passageway out between epidermal cells.

Almost every infection 1 week old has formed stomatal hyphae. Plate 5, *C*, represents such a hypha emerging through a stoma in the

EXPLANATORY LEGEND FOR PLATE 6

- A.—Multinucleate hypha, *a*, in stoma of lower epidermis; 8-day infection. × 1,400.
 B.—Hypha reaching surface between cells of upper epidermis of 9-day infection. × 1,400.
 C.—Hyphae, *a*, *c*, *d*, growing through cells of upper epidermis to the surface; *c* and *d* bearing conidia; germinating conidium at *b*; 7-day infection. × 1,400.
 D.—Conidium at *a* and growth in epidermal cell at *b*, connecting with intracellular hyphae; 8-day infection. × 1,400.
 E.—Nine-day infection. Loose aggregation of hyphae at *a*. Both the mycelium and the host cells invaded by it are dead. × 230.

upper epidermis of the leaf, and *D, b*, shows an occupied stoma of the lower epidermis. The tips of both of these hyphae are dead. Exposed parts of stomatal hyphae are short-lived, for the closing of the stoma pinches the hypha. The hypha can, however, continue to grow out from its base.

Perhaps successive pinchings as the stoma opens and closes have given these stomatal hyphae their moniliform appearance. Another possibility is that these emergent hyphae are cutting off successive cells in some hitherto unsuspected formation of conidia. Plate 5, *E, F, G, H*, from 8-day infections, also suggests conidial formation. In *E*, several hyphae have grown up through the aperture, forcing the stoma wide open. Each bears a terminal knob, or a chain of small rounded cells. In *F*, which shows a longitudinal section of a stoma, the stomatal hyphae have pushed out stubby little branches, two of which, *b, c*, suggest the formation of conidia. Behind one of these hyphae, at *a*, is a small disconnected ovoid spore. *G*, at *a*, again suggests the formation of little ovoid conidia. They vary somewhat in size but are about half the diameter of the sporidia of the rust. In *H*, showing an oblique section through a stoma of the lower epidermis, there are several stomatal hyphae and, at *a*, a spore.

In plate 6, *A*, from the lower epidermis, the outgrowing hypha, *a*, evidently encountered a shut stoma, continued to grow and spread out on the inner face of the stoma, then, when the stoma opened, started to grow down through it. This hypha contains several nuclei. A search was made for free conidia on the surface of the leaf. Only a few have been found but, of course, in fixed material loose cells wash off.

Rarely a mycelial hypha of *Puccinia malvacearum* reaches the surface of the leaf by growing out between ordinary epidermal cells. Plate 6, *B*, shows such a hypha.

Because of the exceptional ability of *Puccinia malvacearum* to grow intracellularly, the hyphae also reach the surface of the host by growing out through epidermal cells (pl. 6, *C*). At *a*, a hypha has grown up to the outer wall of the epidermal cell, and at *c* and *d*, a branching hypha has broken through to the surface. Its shape suggests the formation of conidia. At *b*, is what appears to be a small germinating conidium that has entered the host cell. *D* is more difficult to interpret. At *a* is an empty little cell on the leaf surface, at *b* a small growth inside the host cell in contact with larger hyphae. No connection could be traced between *a* and *b*.

So far as known, *Puccinia malvacearum* produces no spermatogonia. The findings recorded above suggest the interesting possibility, however, that this species is developing an accessory spore form by producing little conidia on the scattered hyphae that grow to the surface of the leaf. If what has been seen so far is representative, comparatively few such conidia are formed. There is always the possibility, however, that at a certain age of the fungus, or at a particular time of day or night, or under optimum weather conditions, conidia would be formed more freely.

Knowing that in some species conidia are formed only during the night (33), material from plants growing out of doors and from leaves placed in a damp chamber indoors was fixed at intervals during the night. Part of this material was used for freehand sections, and the

rest was embedded in paraffin and sectioned. Only an occasional hint of spore formation has been found. Data up to the present give little encouragement to the idea that conidia are ever formed in abundance.

ORIGIN OF BINUCLEATE CELLS AND FORMATION OF THE SORUS

Under the conditions of these experiments, the growing haploid mycelium reaches the lower epidermis of the leaf sometime between the fifth and the seventh days. By the seventh day the infections show a marked difference in development. In some the mycelium is spreading vigorously, the marginal hyphae are healthy, and the sorus is beginning to form just above the lower epidermis. In others the infection is small, the mycelium scanty, the marginal hyphae and the host cells invaded by them are dying, and there is no young sorus. This difference in development can hardly be explained as due to differences in age or vigor of the host leaf or to varying environmental conditions, for within a millimeter of one of these dying infections in a leaf may be a vigorous mycelium making rapid progress toward spore formation. On the eighth day the difference is more pronounced and by the ninth day after inoculation some infections are dead and others close by are growing rapidly and developing young teliospores. Plate 6, *E*, shows a dead 9-day infection. The fungus had formed a loose aggregation of hyphae at *a*, the place for a sorus, but it failed to shape up for spore formation, and now the hyphae are dead and shrunken. Soon after this, the mycelium disappears and the dead host cells appear clear and empty.

A detailed study was made of twenty 8-day infections from a rather heavily infected leaf. Of these, 16 were growing vigorously and 4 were dying. A section-by-section study shows that the four small dying infections are monosporidial in origin. Only one primary hypha can be found in the upper epidermis adjoining each infection. Moreover, these four are still haploid; their cells are uninucleate. Of the 16 vigorous infections, 14 have 2 or more primary hyphae fairly close together, so that the mycelia developing from them are fully confluent and appear macroscopically as 1 infection, and 2 are obviously double, consisting of 2 tangent infections with only the marginal hyphae interlacing. All of the 16 infections have young sori. In the case of tangent doubles, the sorus is apt to be started between the two infections. If the two infections are so far apart that their mycelia do not meet until after several days' growth, the sorus is correspondingly delayed in starting.

The attempt to make a similar study in 9-day material was only partly successful. The proportion of living and dead mycelia is about the same. In some cases it is still possible to prove the multisporidial origin of the living infections, although primary hyphae have usually deteriorated somewhat by the ninth day; it is, however, no longer possible to identify the primary hypha in the dead infections.

In this experiment the monosporidial infections remained small and haploid, failed to initiate a sorus, and soon died. It is not proved, of course, that under no circumstances would an isolated monosporidial infection develop further.

A closer examination of the young multisporidial infection shows that anastomoses take place between hyphae, both in the vegetative mycelium and at the location for the sorus, and that nuclei move

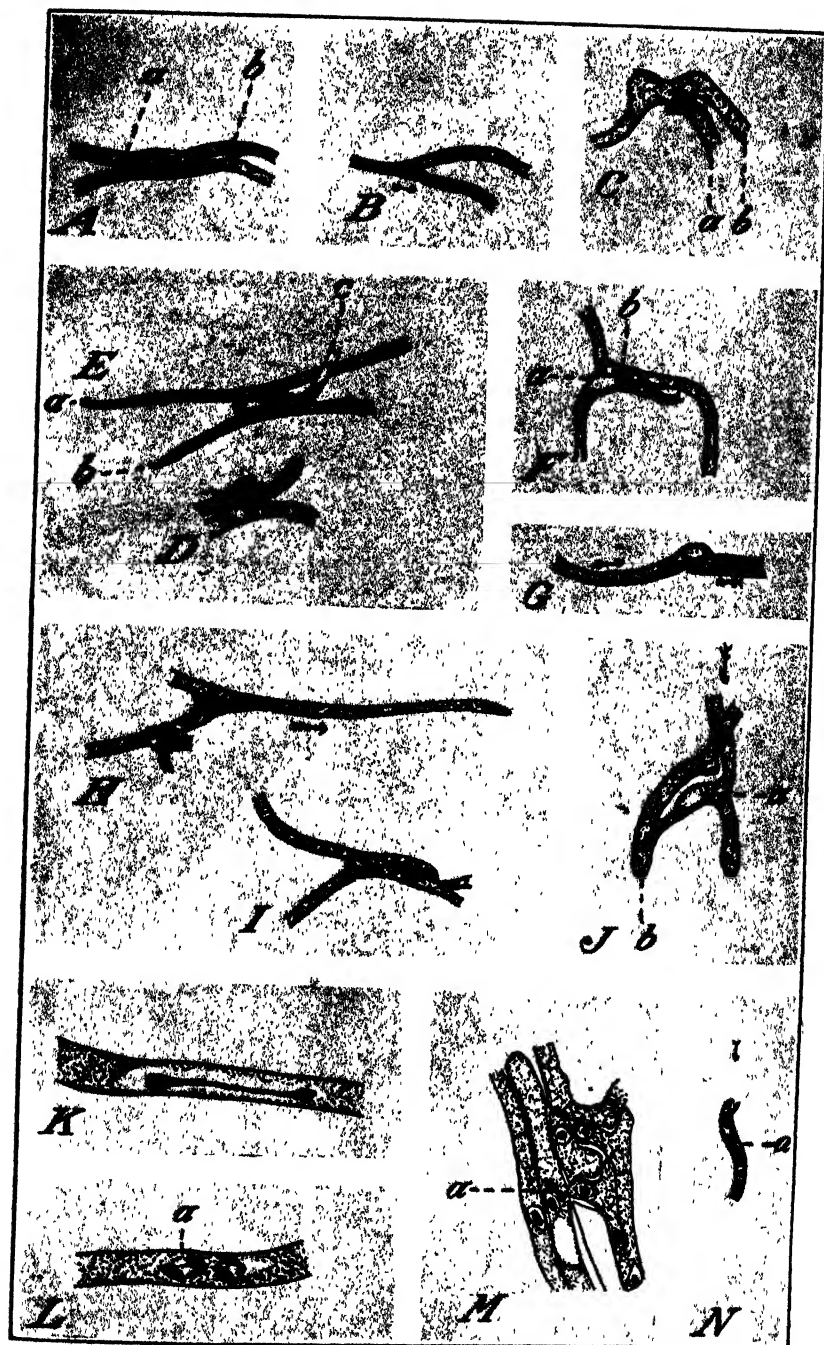
through from one hypha to another. Plate 5, *D*, *a*, and plate 7, *A*, *C*, *D*, *E*, *F*, from 7-day infections, show connections between vegetative hyphae. In plate 5, *D*, at *a*, 2 hyphae growing in opposite directions came close to each other and formed a connecting passageway, giving rise to an H-shape figure. In plate 7, *A*, 2 passageways, at *a* and *b*, formed between parallel hyphae, and a nucleus at *a* is passing over from one hypha to the other. That this is a case of anastomosis and not ordinary branching is seen by comparison with *B*, in which a branch is forming and a nucleus is passing out into the branch. In the one figure there are 4 ends; in the other, 3. In *C* the hypha *a* grew up to and flattened against the hypha *b*; an open passageway formed, and a nucleus is passing through from *b* to *a*. In *D* also, two hyphae are tangent and a nucleus is passing through from one to the other. In *E* two hyphae, *a* and *b*, were growing parallel in the same direction when a third hypha, *c*, grew in and made successive connections with both *a* and *b*. In *F* two hyphae became closely applied to each other. At *a*, in one hypha, is a vacuole; at *b*, in the other hypha, are 2 nuclei. A nucleus may have moved from *a* to *b*.

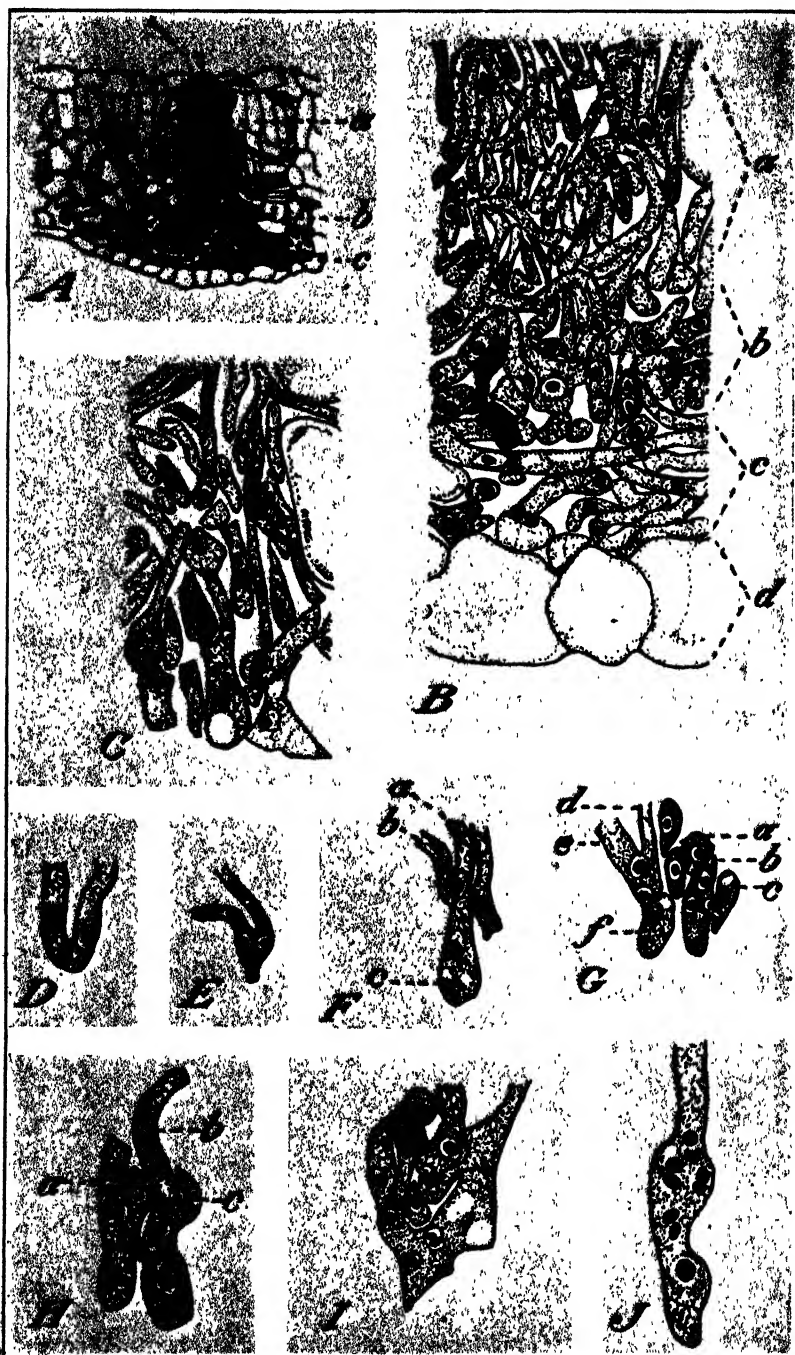
Plate 7, *G*, *H*, *I*, *J*, from older infections, also shows fusions in vegetative hyphae. Two infections started growth so far apart that they did not meet until they were 8 days old. Neither infection had initiated a sorus. At 8 days of age a few marginal hyphae of one made contact with marginal hyphae of the other. Nearly every one of these contacts resulted in a fusion. *G* is typical of these fusions. The tips of these hyphae met and fused, and the nucleus of one hypha passed over into the other hypha. *H*, from another 8-day preparation, shows an unusual configuration, in which apparently the whole growing tip of one hypha fused with another. In *I* the two hyphae were growing in opposite directions when they met, and there is an aggregation of nuclei near the passageway. In *J* the hypha *a* has contributed its nucleus to *b*.

In infections showing these anastomoses and nuclear transfers, binucleate cells are soon found. No general diploidization of the mycelium takes place. Marginal hyphae, if they are not in actual contact with another mycelium, remain haploid. In the central area, however, cells with more than one nucleus become fairly common. Frequent figures, such as plate 7, *K* (more highly magnified than those preceding), showing nuclear locomotion, and figures such as *L*, *a*; *M*, *a*; *N*, *a*, in which only 1 of the 2 nuclei in a cell is dividing, suggest that the transferred nuclei and their progeny may become distributed along a hypha by successive divisions and migrations.

EXPLANATORY LEGEND FOR PLATE 7

- A*.—Two hyphae anastomosed at *a* and *b*. Nucleus passing through at *a*. Seven-day infection. $\times 1,020$.
B.—Branching of hypha, from 7-day infection. $\times 1,020$.
C.—Anastomosis of hyphae, *a* and *b*, with nucleus passing through; 7-day infection. $\times 1,020$.
D.—Anastomosis with nucleus passing through; 7-day infection. $\times 1,020$.
E.—Two parallel hyphae, *a* and *b*, with third hypha, *c*, anastomosing with both; 7-day infection. $\times 1,020$.
F.—Anastomosis of two hyphae, with vacuole at *a*, and two nuclei at *b*, from 7-day infection. $\times 1,020$.
G.—Anastomosis between tips of hyphae and transfer of nucleus; 8-day infection. $\times 1,020$.
H.—Lateral union of two hyphae; 8-day infection. $\times 1,020$.
I.—Anastomosis with aggregation of nuclei; 8-day infection. $\times 1,020$.
J.—Anastomosis with transfer of nucleus from *a* to *b*; from 12-day infection. $\times 1,020$.
K.—Migrating nucleus from 7-day infection. $\times 2,200$.
L.—Binucleate cell with only one nucleus, *a*, dividing; from 7-day infection. $\times 2,200$.
M.—One of two nuclei of a cell at *a* dividing; from 7-day infection. $\times 1,020$.
N.—One of two nuclei at *a* dividing; from 8-day infection. $\times 1,020$.





FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

Plate 8, *A*, represents semidiagrammatically a typical 8-day multi-sporidial infection. The mycelium first invaded a number of palisade cells at *a* and then grew downward. There is very little lateral spread; the mycelium remains strictly localized. The young sorus is taking shape at *b*. The hyphae nearest the lower epidermis, at *c*, will become the buffer cells and are already somewhat impoverished. As a result of earlier mycelial fusions, some of the hyphae are already diploid when they enter the sporogenous layer. *B* and *C* show enlarged details in which many of the cells contain more than one nucleus. *B*, *a*, shows the mycelium above the sporogenous area; *b*, the sporogenous area; *c*, the buffer cells; and *d*, the lower epidermis.

Other hyphae are haploid when they reach the sporogenous layer and fuse there. Plate 8, *D* to *I*, shows details of fusions in the sporogenous area from 8-day infections. In *D*, two hyphae grew down into the sporogenous layer, and their tips met and flattened against each other. In *E*, the wall between the two is dissolved. In *F*, two hyphae, *a* and *b*, grew down, met, and joined, and the fusion cell grew on to *c*. Figures such as this are common. Sometimes the fusions are more irregular. In *G*, apparently 3 cells, *a*, *b*, and *c*, fused; the combination possesses 4 nuclei. Two hyphae, *d* and *e*, fused and grew on downward. Whether *f* represents a cell being cut off or a further fusion is not clear. In *H*, the hyphae *a* and *b* were diploid before reaching the sporogenous layer, but *b*, none the less, has been joined by a third hypha, *c*. In *I*, the connections and fusions are so complicated that several nuclei occur within the limits of the fusion cell. Multinucleate cells in the sporogenous area are not rare; *J* represents an extreme example.

In 9-day infections (if fusions have not been delayed by too great a distance between the component infections) the sorus is larger and young teliospores are forming. Hyphae resulting from earlier mycelial fusions (pl. 9, *A*) can form spores directly. In *C* the one terminal cell of the main hypha, *b*, will become one spore, while a side branch, *a*, will later give rise to another.

Fusion cells (pl. 8, *F*), so abundant in 8-day material, have continued to grow, giving rise to cells such as that shown in plate 9, *B*. At this stage it still is occasionally possible to trace the early history. The two hyphae of plate 9, *B*, *a* and *b*, fused at *c* and the fusion cell grew on to *d*. Usually the fusion cell contains two nuclei, but irregularities are common. Here (*B*) there are two full-grown nuclei plus a pair of small, newly divided nuclei. In *D* appearances suggest that two hyphae, *a* and *b*, met, fused, and then, by adjusted divisions and growth, gave rise to two hyphae, *c* and *d*, both composed of binucleate cells.

Soon after this stage the part of the sorus above the spores becomes a dense pseudoparenchyma (pl. 9, *F*), and it is seldom possible to

EXPLANATORY LEGEND FOR PLATE 8

A.—Diagram of 8-day fertile infection showing invaded palisade cells at *a*, sporogenous area at *b*, and buffer cells at *c*. $\times 160$.

B.—Detail of 8-day fertile infection showing central mycelium, *a*; sporogenous area, *b*; buffer cells, *c*; and lower epidermis, *d*. $\times 1,020$.

C.—Detail from 8-day fertile infection showing cells with more than one nucleus in and above the sporogenous area. $\times 1,020$.

D to *I*.—Details from sporogenous area of 8-day infections. *D*, Two hyphae meeting at tips and about to fuse. *E*, Newly fused hyphae. *F*, Hyphae *a* and *b* fused and the fusion cell grew down to *c*. *G*, Hyphae *a*, *b*, *c*, fused; *d* and *e* fused; *f* may be a further fusion. *H*, Hyphae *a* and *b* already diploid but *b* is joined by *c*. *I*, Much-involved fusions. $\times 1,020$.

J.—Multinucleate cell at sporogenous area; 8-day infection. $\times 2,200$.

trace the origin of the spore-bearing cells in this mass of tightly interwoven hyphae. *E* shows a detail from a 12-day sorus. As new spores continue to form for some time, several stages of spore development can be found side by side. The appearance of a small 15-day open sorus is shown semidiagrammatically in *F*.

Cytological details of the development of the teliospore, the nuclear fusions at maturity, the germination of the spore, the reduction divisions, and the formation of the sporidia are presented in another paper (5) and are not repeated here.

DISCUSSION

It has been assumed that a short-cycle rust without spermogonia, like *Puccinia malvacearum*, is without means of crossing. A closer view of the situation makes this assumption doubtful.

Under natural field conditions, the infections of *Puccinia malvacearum* on a leaf are not all of the same age. As soon as one infection matures its teliospores germinate (weather permitting), and continual crops of sporidia are formed and freed. When a sporidium drops upon a leaf it is apt to roll down into a furrow. Hollyhock and mallow leaves are not smooth; they are rather deeply creased and furrowed at the veins. Sporidia accumulate in these creases and can be seen there by dozens. After a few hours in damp air, the wholesale entrance of these sporidia into epidermal cells can be found. The resulting mycelia are crowded and interlace freely. Moreover, mallow and hollyhock leaves are regularly visited by small spiders and insects; in fact, it is rare to find an infected leaf in the field that is free from such visitors. These help to carry sporidia about.

There are indications, too, that immature infections, too young to be bearing teliospores, can produce small conidia on the scattered hyphae that reach the surface of the leaf by growing into stomatal apertures or growing out through epidermal cells. Only a few such conidia have been found. There is, of course, the possibility that under the right conditions such spores would be formed in greater abundance, but material taken at different times of day and night from plants in ordinary air and in damp chambers, lends small encouragement to such a belief. There is a little evidence that these conidia can germinate and enter the host leaf.

The formation of even a few such conidia early in the development of an individual might be of significance, for they could easily be transferred by wind or by insects, and if by chance placed near another infection, might grow in and make contact with it.

Even in a rust without spermogonia, then, there are frequent contacts between individuals. The means of crossing exist.

This formation of a few scattered conidia in *Puccinia malvacearum* that, theoretically at least, could help to replace in function the absent spermatia, is not unique among higher fungi. De Bary

EXPLANATORY LEGEND FOR PLATE 9

- A*.—Hypha with trinucleate cells from sporogenous area of 9-day infection. $\times 1,020$.
B.—Hyphae *a* and *b* fused at *c* and grew to *d*. Irregular nuclear content. From sporogenous area of 9-day infection. $\times 1,020$.
C.—Diploid hypha branching. Both *a* and *b* will give rise to teliospores. Nine-day infection. $\times 1,020$.
D.—Hyphae *a* and *b* fused and then both grew on as diploid hyphae, *c* and *d*; 9-day infection. $\times 1,020$.
E.—Detail of 12-day infection with growing teliospores of different ages. $\times 1,020$.
F.—Diagram of small 15-day open sorus. $\times 100$.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

(14), in 1887, cites several cases of "doubtful spermatia" in Ascomycetes, and Backus (13) has summed up a variety of irregularities and doubtful cases reported in recent literature. The ability of fungi to produce conidia at unexpected times and places is further illustrated by the production in the diplophase of *Pholiota* (27), of several accessory spore forms of a conidial nature.

In a rust with spermogonia that produce thousands of spermatia in a liquid attractive to insects, there is abundant opportunity for the transfer of cells from one infection to another. A subsidiary spore form like these conidia, under such conditions, would seem to be of less biologic importance to the rust. A hint that such conidia may be formed, however, is given by Rice (30), who mentions and figures, without comment, the occurrence of small "spheres" attached to or lying close to the stomatal hyphae in corn rust.

Hyphae growing out to the leaf surface in young haploid infections of rusts have been known since the days of De Bary (14, p. 278) and have been noted frequently since then (1, 2, 3, 4, 6, 8, 10, 11, 30). The only rust investigated recently in which surface hyphae have not been found is flax rust (7). Further studies may bring to light more cases in which such surface hyphae function as conidiophores.

There is evidence that the isolated monosporidial infection of *Puccinia malvacearum* tends to be retarded in development, remains minute, and under some conditions, at least, remains haploid and dies young without forming a sorus. With the limited data presented here, however, it cannot be taken for granted that an isolated infection never develops a sorus. With other strains of the rust, or other hosts, or other culture conditions, it is possible that a sorus could be formed in an isolated monosporidial infection. Ashworth (12) reports that, in 18 out of 1,000 (i. e., 1.8 percent) single-spore inoculations of *P. malvacearum*, infection took place and sori developed. She assumed that infection did not take place in the other 982 inoculations. So far as stated, however, these were not sectioned, and it is possible that many of the inoculations resulted in minute infections that died without becoming large enough to be visible macroscopically. It is also possible that, while the 18 inoculations resulting in infections with sori were still small, insects transferred conidia or sporidia to them. On this basis, *P. malvacearum* would be heterothallic.

When two mycelia of *Puccinia malvacearum* meet within the leaf, anastomoses form freely. Anastomoses between hyphae of the same mycelium or of two overlapping mycelia of the same species are common in the higher fungi. In species of Hymenomycetes that can be grown in Petri dishes, it is comparatively easy to find such anastomoses (17). It long has been believed that, in rusts also, two mycelia in contact form connections and that nuclei pass through these connections, resulting in the diploidization of both components. Demonstration of this in rusts is difficult, for hyphae wind about in the irregular air spaces of the leaf and in sectioned material appear as comparatively short pieces.

Indirect but none the less conclusive proof of anastomoses in rusts is given by Brown (16). Sporophytic (uredial) and haploid mycelia of *Puccinia helianthi* Schw. were grown side by side on the same leaf. The diploid mycelium contributed to the haploid mycelium nuclei of the kind it lacked and the latter at once developed normal aecia.

In *Puccinia malvacearum*, anastomoses between mycelia are abundant and fairly conspicuous. When two primary hyphae are close together and the first mycelial growth is fairly dense, it is hard to trace connections between the two, although the early presence of binucleate cells in such mycelium suggests that cell fusions have occurred. But when 2 mycelia are farther apart and the first contacts are made between scattered marginal hyphae that have grown toward each other from the 2 infections, nearly every meeting point shows anastomosis.

The presence of anastomoses is not in itself proof that fertilization is taking place. Buller (17) has shown that in *Coprinus* a number of adjoining mycelia may unite into a compound mycelium, forming an intimate network with thousands of connections. This can take place equally well whether the species is homothallic or heterothallic. Combining is of advantage in the absorption and transportation of food materials for the building of a fruit body. Moreover, this type of combination takes place quite independently of sex. Buller says (17, pp. 185-186):

From these observations we are justified in concluding that, in *Coprinus lagopus* and similar Hymenomycetes, any two like mycelia or any two unlike mycelia can unite with one another to form a compound mycelium and that, in general, any one kind of mycelium at one and the same time can combine with any number of other mycelia with which it may come into contact, whatever may be their sexual condition. [He adds, however]: If two mycelia which do not react sexually happen to unite, no association takes place between their nuclei; but, if two mycelia of opposite sex happen to unite, then through the openings made by the first few hyphal fusions nuclei pass from one mycelium into the other mycelium, conjugate pairs of nuclei are soon established in both mycelia, and the mycelial haplophase quickly changes into the diplophase.

In *Puccinia malvacearum*, anastomoses between mycelia are accompanied by the transfer of nuclei. It is not uncommon to find a nucleus in the act of passing through an anastomosis from one hypha to the other. Moreover, shortly after these anastomoses and nuclear transfers, binucleate cells are to be found in the mycelium and a sorus starts promptly. So far as these details also are concerned, it might be supposed that *P. malvacearum* is heterothallic and that this is an ordinary diploidization process.

One further item should be taken into account, however. In heterothallic fungi with 2 sex groups (or compatibility groups, as some writers prefer to call them), roughly 50 percent of the matings are between individuals of the same group and remain sterile, while the other 50 percent are between opposite groups and lead to fertilization and the diploid generation. Present data on *Puccinia malvacearum* are too limited to be conclusive, but, so far as they go, they indicate that all matings lead to cell fusions and the development of a sorus. This recalls the 100-percent interfertility between "geographic" strains in certain Hymenomycetes (23, 25, 31).

Since rusts are obligate parasites and it is not feasible to subdivide infections and plant bits of them in pairs in all possible matings, a genetical analysis cannot be made. Without it, no certain conclusions can be drawn.

It is possible to look at the above data from quite a different point of view. Suppose for the moment that *Puccinia malvacearum* is homothallic. The young sporidium of *P. malvacearum* is uninucleate.

As the spore matures this nucleus regularly divides once. Suppose that as the result of this division one daughter nucleus in the sporidium is (+) and the other (-). Reference to plates 1 and 2 show that both nuclei of a sporidium move into the host and are soon located in separate cells of the primary hypha. Each of these cells gives rise to uninucleate mycelium. On this assumption every monosporidial mycelium would consist of mingled (+) and (-) hyphae in approximately equal numbers. Suppose further that (+) and (-) hyphae grown from the same sporidium react but feebly with each other and in many cases no pairing at all takes place and the mycelium dies without producing a sorus. In a few there is further development and the formation of a sorus. When, on the contrary, two of these monosporidial infections meet within the leaf, anastomoses form, there is an active interchange of (+) and (-) nuclei, and the (+) nuclei of each mycelium meet (-) nuclei of the other. Pairing of nuclei and diploidization can now progress rapidly and a sorus forms in all cases. Of course this theory has not been and perhaps cannot be proved. Its only justification is that it covers the observed facts.

Puccinia malvacearum is a rust with a reduced life cycle, maintaining itself by repeating telial generations. At the time when the other spore forms were lost from the cycle, survival depended on the immediate development of some substitute method of attaining the binucleate phase. Sexual reproduction here is reduced to the simplest terms. There is a complete absence of sex organs and gametes. As in the Hymenomycetes, vegetative hyphae can anastomose and serve as the channel for the interchange of nuclei. Reproduction here is concerned fundamentally with the pairing of nuclei. Gametes can be dispensed with, but the irreducible minimum for sexual reproduction is the presence of two kinds of nuclei, whether the difference between them is labeled a sex factor, compatibility factor, or self-sterility factor (9, 19, 20, 21, 22).

Evidence has accumulated in recent years that the nuclei of higher fungi can migrate freely through a mycelium and even pass through anastomoses from one mycelium to another. Buller (17) has proved that nuclei introduced at one point into a haploid mycelium of *Coprinus* multiply and become rapidly dispersed throughout the whole mycelium. That nuclei of rusts have the same power of locomotion is shown by *Puccinia sorghi* Schw. (8), in which 24 hours after spermatization 60 percent of the cells throughout the mycelium contain more than one nucleus.

The mode of locomotion of a fungus nucleus is unknown. As a beginning toward clearing up this obscure point, the different shapes of nuclei found in rapidly growing tips of hyphae have been arranged in a sequence that can be interpreted as stages in the forward stride of a nucleus. According to this series, a nucleus pushes out a long slender beak, then flows forward into the beak and condenses in the new position, leaving a temporary vacuole in the cytoplasm behind it. This is somewhat in the manner of amoeboid motion. The extreme slenderness of the initial beak helps to explain how a nucleus could pass through a very fine pore in a septum when moving from one cell of a hypha to the next. Buller (17) has figured out the rate at which *Coprinus* nuclei travel when diploidizing a haploid mycelium. A nucleus at full speed moves ahead a distance equal to its own diam-

eter every 6 seconds. In *Puccinia malvacearum*, the beak on a nucleus in motion becomes about three times as long as the body of the nucleus, and the length of the beak determines the length of the forward stride. If a rust nucleus can travel as fast as a *Coprinus* nucleus, a single stride would take about 18 seconds. If one allows for a pause between strides (table 1), the advance itself must take less than 18 seconds.

In *Puccinia sorghi*, fertilization is followed by wide-spread diploidization of the mycelium. In *P. malvacearum* binucleate cells are found principally in the neighborhood of the sorus. The cells of marginal hyphae, except when in direct contact with another mycelium, remain uninucleate. Correlated with this difference between the two species is the fact that one mycelium of *P. sorghi* produces many aecia in successive marginal circles, while *P. malvacearum* produces only one central sorus.

SUMMARY

Puccinia malvacearum Bert. is a short-cycle rust of hollyhocks and mallows maintaining itself by repeated telial generations. Spermatogonia, aecia, and uredia are unknown.

Teliospores can germinate as soon as formed, giving rise to a promycelium bearing four binucleate sporidia. The sporidia germinate at once and their germ tubes enter the leaf and give rise to uninucleate mycelium, which is both intercellular and intracellular.

Nuclear locomotion incidental to vegetative growth and also the longer nuclear migrations during diploidization have been studied. In making a forward stride, a nucleus pushes out a long slender beak, then flows forward into the beak and condenses in the new position, leaving a temporary vacuole in the cytoplasm behind it.

Hyphae reach both the upper and the lower surface of the host leaf by growing into stomata and by growing out through and, more rarely, between the other epidermal cells. There is some evidence that these surface hyphae produce small ovoid conidia about half the size of sporidia.

The isolated monosporidial infection is retarded in growth and may die without developing a sorus.

Whenever two monosporidial mycelia meet within the leaf anastomoses form and a nuclear interchange takes place, followed by localized diploidization and the development of a sorus. Some hyphae are already diploid when they reach the sporogenous area of the sorus and can give rise directly to teliospores. Others are haploid and pair in the sorus before forming teliospores.

LITERATURE CITED

- (1) ALLEN, R. F.
1930. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA GRAMINIS.
Jour. Agr. Research 40: 585-614, illus.
- (2) ———
1932. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA TRITICINA.
Jour. Agr. Research 44: 733-754, illus.
- (3) ———
1932. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA CORONATA.
Jour. Agr. Research 45: 513-541, illus.
- (4) ———
1933. FURTHER CYTOLOGICAL STUDIES OF HETEROTHALLISM IN PUCCINIA GRAMINIS. Jour. Agr. Research 47: 1-16, illus.

- (5) ALLEN, R. F.
1933. A CYTOLOGICAL STUDY OF THE TELIOSPORES, PROMYCELIA, AND SPORIDIA IN PUCCINIA MALVACEARUM. *Phytopathology* 23: 572-586, illus.
- (6) ———
1933. THE SPERMATIA OF CORN RUST, PUCCINIA SORGH. (*Phytopath. Note*) *Phytopathology* 23: 923-925, illus.
- (7) ———
1934. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN FLAX RUST. *Jour. Agr. Research* 49: 765-791, illus.
- (8) ———
1935. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA SORGH. *Jour. Agr. Research* 49: 1047-1068, illus.
- (9) AMES, L. M.
1934. HERMAPHRODITISM INVOLVING SELF-STERILITY AND CROSS-FERTILITY IN THE ASCOMYCETE, PLEURAGE ANSERINA. *Mycologia* 26: 392-414, illus.
- (10) ANDRUS, C. F.
1931. THE MECHANISM OF SEX IN UROMYCES APPENDICULATUS AND U. VIGNAE. *Jour. Agr. Research* 42: 559-587, illus.
- (11) ———
1933. SEX AND ACCESSORY CELL FUSIONS IN THE UREDINEAE. *Jour. Wash. Acad. Sci.* 23: 544-557, illus.
- (12) ASHWORTH, D.
1931. PUCCINIA MALVACEARUM IN MONOSPORIDIAL CULTURE. *Brit. Mycol. Soc. Trans.* 16: 177-202, illus.
- (13) BACKUS, M. P.
1934. INITIATION OF THE ASCOCARP AND ASSOCIATED PHENOMENA IN COCCOMYCES HIEMALIS. *Boyce Thompson Inst. Plant Research Contrib.* 6: 339-379, illus.
- (14) BARY, A. DE
1887. COMPARATIVE MORPHOLOGY AND BIOLOGY OF THE FUNGI, MYCETOOZOA AND BACTERIA. Authorized English transl. by H. E. F. Garnsey... rev. by I. B. Balfour. 525 pp., illus. Oxford.
- (15) BLACKMAN, V. H., and FRASER, H. C. I.
1906. FURTHER STUDIES ON THE SEXUALITY OF THE UREDINEAE. *Ann. Bot. [London]* 20: [35]-48, illus.
- (16) BROWN, A. M.
1932. DIPLOIDISATION OF HAPLOID BY DIPLOID MYCELIUM OF PUCCINIA HELIANTHI SCHW. *Nature [London]* 130: 777, illus.
- (17) BULLER, A. H. R.
1931. RESEARCHES ON FUNGI. VOLUME IV. FURTHER OBSERVATIONS ON THE COPRINI TOGETHER WITH SOME INVESTIGATIONS ON SOCIAL ORGANIZATION AND SEX IN THE HYMENOMYCETES. 329 pp., illus. London, New York [etc.]
- (18) CRAIGIE, J. H.
1927. DISCOVERY OF THE FUNCTION OF THE PYCNIA OF THE RUST FUNGI. *Nature [London]* 120: 765-767, illus.
- (19) DODGE, B. O.
1932. THE NON-SEXUAL AND THE SEXUAL FUNCTIONS OF MICROCONIDIA OF NEUROSPORA. *Bull. Torrey Bot. Club* 59: 347-360, illus.
- (20) DOWDING, E. S.
1931. THE SEXUALITY OF THE NORMAL, GIANT, AND DWARF SPORES OF PLEURAGE ANSERINA (CES.), KUNTZE. *Ann. Bot. [London]* 45: 1-14, illus.
- (21) ———
1931. THE SEXUALITY OF ASCOBOLUS STERCORARIUS AND THE TRANSPORTATION OF THE OIDIA BY MITES AND FLIES. *Ann. Bot. [London]* 45: [621]-637, illus.
- (22) DRAYTON, F. L.
1934. THE SEXUAL MECHANISM OF SCLEROTINIA GLADIOLI. *Mycologia* 26: 46-72, illus.
- (23) HANNA, W. F.
1925. THE PROBLEM OF SEX IN COPRINUS LAGOPUS. *Ann. Bot. [London]* 39: [431]-457, illus.

- (24) JACKSON, H. S.
1931. PRESENT EVOLUTIONARY TENDENCIES AND THE ORIGIN OF LIFE CYCLES IN THE UREDINALES. *Mem. Torrey Bot. Club* 18: 1-108, illus.
- (25) KNIEP, H.
1923. ÜBER ERBLICHE ÄNDERUNGEN VON GESCHLECHTSFACTOREN BEI PILZEN. *Ztschr. Induktive Abstam. u. Vererbungslehre* 31: [170]-183.
- (26) LINDFORS, T.
1924. STUDIEN ÜBER DEN ENTWICKELUNGSVERLAUF BEI EINIGEN ROST-PILZEN AUS ZYTOLOGISCHEN UND ANATOMISCHEN GESICHTS-PUNKTEN. *Svensk Bot. Tidskr.* 18: 1-84, illus.
- (27) MARTENS, P., and VANDENDRIES, R.
1932. LE CYCLE CONIDIEN, HAPLOÏDE ET DIPLOÏDE, CHEZ *PHOLIOTA AURIVELLA*. *Cellule* 41: [337]-388, illus.
- (28) MOREAU, MME. F.
1914. LES PHÉNOMÈNES DE LA SEXUALITÉ CHEZ LES URÉDINÉES. *Botaniste* 13: [145]-284, illus.
- (29) OLIVE, E. W.
1911. NUCLEAR CONDITIONS IN CERTAIN SHORT-CYCLED RUSTS. (Abstract) *Science (n. s.)* 33: 194.
- (30) RICE, M. A.
1933. REPRODUCTION IN THE RUSTS. *Bull. Torrey Bot. Club* 60: 23-54, illus.
- (31) VANDENDRIES, R.
1923. NOUVELLES RECHERCHES SUR LA SEXUALITÉ DES BASIDIOMYCÈTES. *Bull. Soc. Roy. Bot. Belg.* 56: [73]-97, illus.
- (32) WERTH, E., and LUDWIGS, K.
1912. ZUR SPOREN-BILDUNG BEI ROST UND BRANDPILZEN. (*USTILAGO ANTHEARUM* FRIES UND *PUCCINIA MALVACEARUM* MONT.) *Ber. Deut. Bot. Gesell.* 30: 522-528, illus.
- (33) WESTON, W. H., JR.
1924. NOCTURNAL PRODUCTION OF CONIDIA BY *SCLEROSPORA GRAMINICOLA*. *Jour. Agr. Research* 27: 771-784, illus.

PHYSIOLOGIC SPECIALIZATION OF *MELAMPSORA LINI* ON *LINUM USITATISSIMUM*¹

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INTRODUCTION

Melampsora lini (Pers.) Lév., the fungus that causes rust of cultivated flax, *Linum usitatissimum* L., occurs in all the major seed flax- and fiber-flax-producing areas of the world. Injury to the seed crop results from a reduction in the amount of foliage and from the utilization by the fungus of some of the food of the host plant. In addition to interfering with the normal photosynthetic metabolism of the host plant, rust on fiber flax injures the quality by causing breakage of fibers and preventing normal retting, thus producing what is known as "measly" fibers (23).³ Consequently a small amount of infection may injure the quality of a fiber-flax crop, while it is only under epidemic conditions that the rust may be destructive to seed flax. Only occasionally is flax rust very destructive in Minnesota and the Dakotas, where most of the domestic flaxseed is grown. However, it sometimes occurs in epidemic form in these areas, and Brentzel⁴ and Hart (9) have reported losses ranging from a trace to 100 percent.

Measures that have been recommended for the control of flax rust include early sowing, thorough cleaning of the seed, crop rotation, use of high, well-drained land, destruction or removal of infected straw, and the use of resistant varieties. Henry (10) considered the last-named method the most promising and found rust immunity to be inherited independently of morphologic type or wilt resistance.

In a program to develop disease-resistant varieties of crop plants it is essential to know whether more than one physiologic form of the pathogen occurs and, if so, the varietal host range, distribution, and stability of each form. The object of the investigation herein reported was to obtain this information in regard to *Melampsora lini*.

HISTORICAL REVIEW

Persoon (18) described a rust fungus on *Linum catharticum* L. and *L. usitatissimum* in 1801 and named it *Uredo miniata* β *lini*. In 1847 Lévillé (15) transferred it to the genus *Melampsora*, calling it *M. lini*. Buchheim (5) states that Körnicke, in 1865, decided that the rust on cultivated flax, *L. usitatissimum*, was physiologically

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³ Reference is made by number (italic) to Literature Cited, p. 836.

⁴ FROMME, F. D. RUST CAUSED BY *MELAMPSORA LINI* (PERS.) DESM. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bull. Sup. 15: 168-169, illus. 1921. [Mimeographed.]

distinct from that on *L. catharticum*, and called the former *M. lini* var. *liniperda*. Since then the host range of rusts occurring on different species of *Linum* has been studied by a number of investigators. Fuckel (8), Palm (17), and Buchheim (5) concluded from cross-inoculation studies that rust on cultivated flax was physiologically and, in some cases, morphologically distinct from that on certain wild flaxes. Arthur (2), in demonstrating that *M. lini* was eu-autoecious, obtained pycnia and aecia on *L. lewisii* Pursh and *L. usitatissimum* by inoculation with teliospores produced on the latter species. Hart (9) was unable, however, to infect *L. lewisii* with urediospores from *L. usitatissimum* but did secure successful infection of *L. rigidum* Pursh. Lafferty, Rhynehart, and Pethybridge (14) obtained slight but abnormal infection of *L. angustifolium* Huds. with urediospores from *L. usitatissimum*. Henry (11) found that certain strains of *L. usitatissimum*, *L. angustifolium*, *L. usitatissimum* var. *crepitans* Bönningh., *L. rigidum*, and *L. sulcatum* Riddell were completely susceptible to *M. lini* collected on *L. usitatissimum*, and that certain strains of *L. usitatissimum* and *L. angustifolium*, as well as all tested strains of *L. perenne* L., *L. austriacum* L., *L. grandiflorum* Desf., *L. flavum* L., and *L. catharticum*, were immune.

Hart (9) obtained urediospore collections of *Melampsora lini* on *Linum usitatissimum* from a number of localities in the United States and Canada but could not demonstrate the occurrence of physiologic forms from their reactions on a susceptible and an immune variety of cultivated flax. Henry (10) suspected the occurrence of physiologic forms of rust on cultivated flax but did not demonstrate their existence. He found that four strains of flax that had been selected by Dorst (7) for their resistance to rust in the Netherlands were uniformly susceptible when grown in Minnesota.

MATERIAL AND METHODS

RUST COLLECTIONS AND INOCULATION PROCEDURE

The rust collections used in these studies were made in the field in 1931, 1932, 1933, and 1934. Because of drought, rust was of little economic importance during this period in the flaxseed-producing area of the United States, but a scattered infection occurred throughout Minnesota and the eastern parts of North Dakota and South Dakota. The pathogenicity of 99 uredial collections was studied during these 4 years. Most of the field collections contained but a few uredia and each collection was increased on a susceptible variety in the greenhouse. The urediospores thus obtained were collected in glass vials and stored at 4° C., at which temperature they retained their viability from 2 to 4 months. Pathogenicity tests were made by inoculating approximately twelve 30-day-old plants of each variety with the increased supply of urediospores by dusting the spores from a camel's-hair brush onto the leaves and terminal bud. The inoculated plants were placed in moist chambers for 24 hours at a temperature of 14° to 16° C. and sprayed periodically with an atomizer. They were then removed to rust compartments in a greenhouse kept at a temperature of approximately 20° C. During the winter months a light day of 16 hours was maintained by supplementing daylight with artificial illumination. Final readings were made 10 to 15 days after inoculation, depending on the effect on pustule formation of temperature and light conditions during the period of incubation.

DIFFERENTIAL HOSTS

Although the existence of physiologic forms of *Melampsora lini* on common flax had been suspected by Henry (10) prior to the present studies, it had not been proved nor had suitable differential hosts been discovered. The work of previous investigators on physiologic specialization in the rusts and other fungi had indicated that differential hosts most likely would be obtained from resistant varieties and those possessing diverse morphologic characters. In trying to discover potential differentials, the reaction of 50 varieties of flax to the 36 rust collections made in 1931 and 1932 was studied. These varieties had been chosen because they represented diverse morphologic types or had been reported as resistant to rust.

In these tests it was discovered that many varieties reported as resistant in field trials showed no resistance in greenhouse inoculation tests. Furthermore, the reaction of individual plants of varieties possessing some resistance was extremely variable. Consequently, it was necessary to develop lines pure for rust reaction from those varieties that gave indications of possessing differential potentialities. This was accomplished by plant selection. It was found, for example, that two-thirds of the plants of Williston Golden (C. I.⁵ 25) showed a high degree of resistance when inoculated with form 1, and that the remaining third showed extreme susceptibility. All plants of this variety were susceptible to form 2. Those plants that showed resistance to form 1 were grown to maturity in the greenhouse, and seed from each plant was increased in the field nursery. The progeny of each plant selection was harvested separately and tested for rust reaction to form 1. Many of the progenies thus obtained continued to segregate for resistance and susceptibility, but several were found to be pure for resistance. One of the latter was subsequently selected as a differential strain for future work. Similarly, it was found that 90 percent of the plants of Buda (C. I. 270) were resistant to form 1, while all plants of this variety were susceptible to form 4. A strain of this variety that was pure for resistance to form 1 was developed by the method used in obtaining the resistant strain of Williston Golden.

The reaction of the differential strains of Williston Golden and Buda to the 36 collections made in 1931 and 1932 indicated that at least 5 physiologic forms of flax rust were present. Monosporous uredial cultures were obtained from each of the 5 forms that appeared to be physiologically distinct. In every instance the pathogenicity of the monosporous culture was identical with that of the original collection. The pathogenicity of these 5 monosporously derived forms to 115 additional varieties was determined in 1932. By these tests and the subsequent increase of certain plant selections, a line pure for rust reaction was obtained from each of 12 additional varieties of flax possessing differential potentialities. Four of these, Samarkan (C. I. 514), Abyssinian (C. I. 511), and Akmolinsk (C. I. 515 and 520), although varying somewhat in the degree of susceptibility, reacted similarly to the different forms. Another strain of Abyssinian (C. I. 701) was resistant to the same forms as were the varieties mentioned above but was also resistant to some of the rust forms to which these varieties were susceptible. Selections from Redwing (C. I. 499) possessed varying degrees of resistance but reacted in general as did the differential strain from Buda. The selection from the fiber flax, J. W. S.

⁵ C. I. refers to accession number of the Division of Cereal Crops and Diseases.

(C. I. 708), was either highly resistant or very susceptible to the different forms. The selection from Argentina (C. I. 705) was particularly sensitive to changes in light conditions. This change in reaction was especially noticeable in those forms to which this selection was more or less resistant. The number and size of pustules and the abundance of spores in the pustules were greater in clear weather than in cloudy weather. However, the change in reaction was not sufficient to nullify the value of this variety as a differential. The selections from Diadem (C. I. 321), "very pale blue crimped" (C. I. 647), and Williston Brown (C. I. 803), a brown-seeded selection, probably a hybrid or a mixture, found in a sample of Williston Golden (C. I. 25), were susceptible to most forms of rust but were sufficiently resistant to other forms to be of value as differentials. The selection from Kenya (C. I. 709) was resistant to most forms but semiresistant to some.

HOST REACTION AND TYPES OF INFECTION

The classes of host reaction and infection types used by Stakman and Levine (20) served as a guide for formulating the classification used in these studies. Apparently, flax varieties give more diverse manifestations of resistance to rust infection than do wheat varieties, and the classification of host reaction was modified accordingly. The principal types of reaction of flax varieties to rust infection are described below and shown in plate 1.

<i>Classes of host reaction</i>	<i>Types of rust infection</i>
Nearly immune-----	(0) No uredia developed; hypersensitive flecks or necrotic lesions usually present, but sometimes there is no evidence of infection.
Resistant-----	(1) Uredia minute to small, rarely extending through the leaf, usually distinct and scattered in chlorotic to necrotic areas, but in some cases pustule formation is not accompanied by either chlorosis or necrosis of the surrounding leaf tissue.
	(2) Uredia small to medium, associated with distinct necrosis of the leaf; may be scattered or may form crustlike aggregations in necrotic areas; if isolated, usually are surrounded by a necrotic zone.
Semiresistant-----	(3—) Uredia variable; heavily inoculated areas necrotic, with arrested pustule development; medium to large pustules produced in healthy tissue adjacent to necrotic areas; pustules on stem and cotyledons small but with no evidence of hypersensitiveness.
Moderately susceptible--	(3) Uredia medium to large; well developed but not compound; usually extending through the leaf to both surfaces; development somewhat retarded in heavily infected portions of the leaves; tissues adjacent to uredia may become more or less chlorotic as the pustules mature.
Highly susceptible-----	(4) Uredia large and, if isolated, usually compound, extending through leaf to both surfaces; at first leaves show little chlorosis but later may become chlorotic and die prematurely.

Hard and fast rules for pustule classification cannot be followed. The types of infection exhibited by a variety depend upon such factors as light intensity, length of day, temperature, and age and vigor of the host plant, as well as upon the pathogenic properties of the rust form involved. All ranges of reaction between immunity and complete susceptibility occur, and whether the borderline cases fall into one type of host reaction or into another often depends upon the arbitrary judgment of the observer.

There appear to be at least two distinct forms of resistance in flax. In some varieties resistance is manifested by a reduction in the size of the individual uredia, together with more or less chlorosis, and the eventual premature death of the infected areas of the leaf. In other varieties resistance is manifested by necrosis of the infected region, coupled with more or less repressed pustule formation. Varieties with host reactions of type 1 show different degrees of the first form of resistance; those with reactions of type 2, different degrees of the second form. The extreme cases of both kinds of resistance would fall in the group having reactions of type 0, in which chlorosis or necrosis of the infected portions of the leaves occurs without pustule formation. Reactions of type 1— include those in which chlorosis of the infected leaves occurs but in which the minute uredia do not break through the epidermis; reactions of type 1 include those in which the small uredia, usually borne in chlorotic areas of the leaves, break through the epidermis; and reactions of type 1+ include those in which small, erumpent uredia are produced, with but slight, if any, chlorosis of the surrounding leaf tissue. Reactions of type 2— are characterized by the production of small aggregations of rudimentary uredia in necrotic areas of the leaves; those of type 2, by the production of small to mid-sized uredia surrounded by a sharp necrotic zone; and those of type 2+, by a crustlike structure resulting from the aggregation of uredia produced in necrotic areas of the leaf. Each form of resistance, as represented by types 1 and 2, covers a range of host reaction extending from near immunity to the borderline of susceptibility. Consequently, one form parallels the other in degree of resistance and neither can be considered, in this respect, subordinate to the other.

No cases of a truly mesothetic type of rust infection, comparable to those occurring in wheat (20), oats (3), and rye (6), were observed on flax. However, there were cases on the borderline of resistance and susceptibility in which monosporous rust cultures produced both necrosis and normal uredia. Since these uredia were well developed and not always produced in or surrounded by necrotic areas, infections of this type were classified as the 3— type and the varietal reaction as semiresistant.

EXPERIMENTAL RESULTS

DIFFERENTIATION OF PHYSIOLOGIC FORMS

Fourteen physiologic forms of *Melampsora lini* infecting *Linum usitatissimum* have been differentiated by the use of seven varieties of cultivated flax. With the study of additional rust collections and the discovery and purification of still other differential varieties, it is probable that more forms will be distinguished. Tests already conducted indicated that additional forms may have been present in the 99 rust collections studied. It was thought inadvisable, however, to establish new forms based on these minor differences, even though they were consistently obtained.

These 14 physiologic forms have been differentiated by the reaction of the selected strain from each of the seven varieties used in the following key. The reactions of each of these seven varieties are given in table 1. In this table the reactions of two additional varieties also are given for additional information and for possible use in differentiating forms not yet studied.

KEY

Buda resistant.	
Williston Golden resistant.	
Akmolinsk resistant.	
Williston Brown semiresistant to resistant.....	Form 10
Williston Brown susceptible.....	Form 1
Akmolinsk susceptible.	
J. W. S. resistant.....	Form 5
J. W. S. susceptible.....	Form 7
Williston Golden susceptible.	
"Very pale blue crimped" resistant.....	Form 11
"Very pale blue crimped" susceptible.....	Form 6
Buda semiresistant.	
Williston Golden resistant.....	Form 3
Williston Golden susceptible.....	Form 14
Buda susceptible.	
Williston Golden resistant.	
Akmolinsk resistant.	
J. W. S. resistant.....	
Kenya resistant.....	Form 4
Kenya semiresistant.....	Form 12
J. W. S. susceptible.....	Form 13
Akmolinsk susceptible.....	Form 8
Williston Golden susceptible.	
J. W. S. resistant.....	Form 2
J. W. S. susceptible.....	Form 9

TABLE 1.—Reactions of pure-line selections from 9 differential varieties of *Linum usitatissimum* to 14 physiologic forms of *Melampsora lini* as determined in greenhouse tests at Fargo, N. Dak., 1935¹

Physiologic form	Types of rust infection and reactions of differential hosts								
	Buda (C. I. 270)			Williston Golden (C. I. 25)			Williston Brown (C. I. 803)		
	Infection type		Host reaction	Infection type		Host reaction	Infection type		Host reaction
	Range	Predominant		Range	Predominant		Range	Predominant	
1.....	0 to 1+	1-	R+	0 to 1.....	1	R+	3.....	3	S
2.....	1 to 3.....	3	S-	3 to 4.....	3	S+	3- to 3.....	3	S
3.....	1- to 3.....	3-	SR	1- to 1.....	1	R+	3- to 3.....	3	S
4.....	3 to 4.....	3	S+	1- to 1.....	1	R+	3.....	3	S
5.....	0 to 1-.....	0	R+	1 to 1+.....	1+	R-	3.....	3	S
6.....	1- to 1.....	1	R+	3 to 4.....	3	S+	3.....	3	S
7.....	1 to 1+.....	1	R+	1- to 1.....	1	R+	3- to 3.....	3-	S-
8.....	3.....	3	S	1- to 1.....	1	R+	3.....	3	S
9.....	1+ to 3.....	3	S-	1+ to 3.....	3	S-	3.....	3	S
10.....	0.....	0	I	0 to 1.....	0	R+	1 to 3-.....	2	SR
11.....	0 to 1-.....	0	R+	3 to 4.....	3	S+	3.....	3	S
12.....	1 to 3.....	3	S-	1 to 1+.....	1	R-	3.....	3	S
13.....	1+ to 3.....	3	S-	1 to 1+.....	1+	R-	3.....	3	S
14.....	1 to 3-.....	3-	SR	3.....	3	S	3.....	3	S

¹ Plus and minus signs indicate somewhat greater or lesser amount of rust than the nearest figure representing the infection type, and the letters signify the following: R, resistant; S, susceptible; SR, semi-resistant; and I, immune.

EXPLANATORY LEGEND FOR PLATE 1

Infection types produced by *Melampsora lini* on selected varieties of cultivated flax, *Linum usitatissimum*.
 A, Type 0. No uredia produced, but chlorotic to necrotic areas formed in inoculated portions of the leaves. Akmolinsk, C. I. 515, inoculated with form 4.

B, Type 1. Small uredia formed usually in association with chlorosis of the surrounding leaf tissue. Williston Golden, C. I. 25, inoculated with form 1.

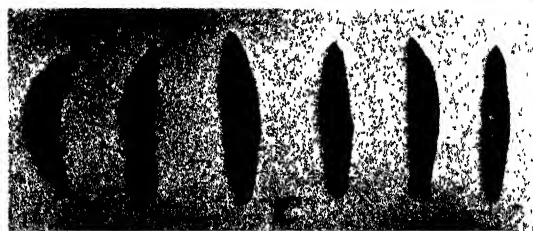
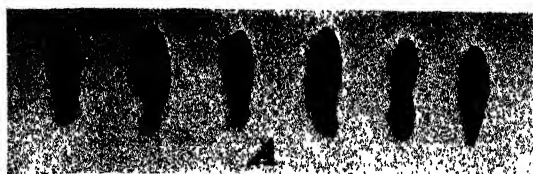
C, Type 2. Small to medium uredia of subnormal development surrounded by sharply defined necrotic areas. Williston Brown, C. I. 803, inoculated with form 10.

D, Type 3-. Isolated uredia, large and well developed but not compound, surrounded by a chlorotic area which may become necrotic; heavily infected portions of the leaves are chlorotic with arrested uredial development. Buda, C. I. 270, inoculated with form 3.

E, Type 3. Uredia large and well developed but not compound; considerable chlorosis in heavily infected areas, but uredial development only slightly retarded. Williston Golden, C. I. 25, inoculated with form 2.

F, Type 4. Uredia large and, if isolated, compound with but slight chlorosis except in the older infections. Bison, C. I. 390, inoculated with form 2.

A to F, $\times 1.5$; F, natural size.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

TABLE 1.—*Reactions of pure-line selections from 9 differential varieties of Linum usitatissimum to 14 physiologic forms of Melampsora lini as determined in greenhouse tests at Fargo, N. Dak., 1935—Continued.*

Physiologic form	Types of rust infection and reactions of differential hosts								
	Akmolinsk (C. I. 515)			J. W. S. (C. I. 708)			"Very pale blue crimped" (C. I. 647)		
	Infection type		Host reaction	Infection type		Host reaction	Infection type		Host reaction
	Range	Predominant		Range	Predominant		Range	Predominant	
1.....	0 to 1—	0	R+	0.....	0	I	3— to 3.....	3	S
2.....	0 to 1—	0	R+	0.....	0	I	3— to 3.....	3	S
3.....	3 to 4.....	3	S+	0.....	0	I	3— to 3.....	3	S
4.....	0 to 1.....	1—	R+	0.....	0	I	3— to 3.....	3	S
5.....	3 to 4.....	3	S+	0.....	0	I	1 to 3—	3—	SR
6.....	0 to 1.....	1—	R+	0.....	0	I	3— to 3.....	3	S
7.....	3 to 4.....	3	S+	3.....	3	S	2 to 3.....	3	S—
8.....	3 to 4.....	3	S+	0.....	0	I	3.....	3	S
9.....	0.....	0	I	3 to 4.....	3	S+	3.....	3	S
10.....	0.....	0	I	0.....	0	I	3— to 3.....	3	S
11.....	0 to 1.....	1—	R+	0.....	0	I	0.....	0	I
12.....	0 to 1.....	0	R+	0.....	0	I	3— to 3.....	3	S
13.....	0.....	0	I	3 to 4.....	3	S+	3— to 3.....	3	S
14.....	0.....	0	I	0.....	0	I	2 to 3.....	3	S—

Physiologic form	Types of rust infection and reactions of differential hosts								
	Kenya (C. I. 700)			Argentina (C. I. 705)			Abyssinian (C. I. 701)		
	Infection type		Host reaction	Infection type		Host reaction	Infection type		Host reaction
	Range	Predominant		Range	Predominant		Range	Predominant	
1.....	0 to 1.....	1—	R+	1— to 3—	2+	SR	0.....	0	I
2.....	1 to 2.....	2	R+	2 to 3—	2+	SR	0.....	0	I
3.....	2+ to 3—	2+	SR	1— to 3—	2+	SR	1.....	1	R+
4.....	2— to 2+..	2—	R	2 to 3—	2+	SR	0.....	0	I
5.....	0 to 2.....	0	R+	2 to 3—	2+	SR	1 to 1+..	1	R+
6.....	0 to 2+..	2—	R	2 to 3—	2+	SR	0.....	0	I
7.....	0 to 2.....	2—	R+	3— to 3.....	3	S	1 to 1+..	1	R+
8.....	2 to 3—	2+	SR	2 to 3—	2+	SR	3.....	3	S
9.....	2— to 2+..	2—	R	2+ to 3—	2+	SR	0.....	0	I
10.....	0 to 1—	0	R+	0 to 1.....	0	R+	0.....	0	I
11.....	0.....	0	I	2 to 3—	2+	SR	0.....	0	I
12.....	2+ to 3—	2+	SR	2+ to 3—	2+	SR	0.....	0	I
13.....	2+ to 3—	2+	SR	2+ to 3—	2+	SR	0.....	0	I
14.....	0 to 2.....	2—	R+	0 to 2+..	2	R	0.....	0	I

DESCRIPTION OF FORMS

Form 1.—Differential selections of J. W. S. and Abyssinian C. I. 701 nearly immune; Buda, Williston Golden, Akmolinsk, and Kenya highly resistant; Argentine C. I. 705 semiresistant; Williston Brown susceptible. Under optimum rust conditions, Williston Golden developed infection types 1 to 1+; under suboptimum conditions it showed pronounced chlorosis and subsequent necrosis of inoculated leaf areas and an occasional pustule of type 1— to 1. Typical response of Buda, Akmolinsk, and Kenya, formation of chlorotic to necrotic areas with an occasional pustule of type 1—. Response of Argentine C. I. 705 variable; uredia usually aggregated in necrotic areas; under suboptimum rust conditions uredia dry prematurely and spore production is sparse; under optimum rust conditions uredia well developed and sporulation abundant.

Form 2.—Buda susceptible; Williston Golden very susceptible, and J. W. S. nearly immune. Uredia on Buda medium in size, numerous, sporulation abundant; stems and cotyledons show no resistant reaction; heavily infected leaves become chlorotic and prematurely dry.

Form 3.—Buda semiresistant; Williston Golden highly resistant; Akmolinsk very susceptible; Kenya semiresistant. Buda, uredia type 3—; variable, heavily inoculated areas necrotic with arrested pustule development, normal uredia in adjacent tissue; uredia on stem and cotyledons small but otherwise normal.

Form 4.—Buda very susceptible; Williston Golden and Akmolinsk highly resistant; Kenya resistant; J. W. S. nearly immune. Infection on Buda normal, uredia large, rarely compound, sporulation abundant, but under suboptimum conditions Buda less susceptible to form 4 than Akmolinsk to form 3 or Williston Golden to form 2. Kenya, heavily inoculated areas chlorotic to necrotic, pustules rudimentary, usually in necrotic areas at leaf margins.

Form 5.—Resistance of Buda and Kenya differentiates this form from form 3. Williston Golden slightly more susceptible than to form 3; Akmolinsk very susceptible; and J. W. S. nearly immune from both forms.

Form 6.—Buda highly resistant; Williston Golden very susceptible. Susceptibility of the "very pale blue crimped" (C. I. 647) differentiates this form from form 11.

Form 7.—Buda, Williston Golden, and Kenya very resistant; Akmolinsk very susceptible; and J. W. S. susceptible; Williston Brown semiresistant to moderately susceptible, uredia large, fewer than normal, and surrounding tissue dries prematurely. The only form producing a susceptible reaction on Argentine C. I. 705 under suboptimum conditions.

Form 8.—J. W. S. nearly immune; Williston Golden highly resistant; Buda susceptible; and Akmolinsk very susceptible. The only form that produces a susceptible reaction on Abyssinian C. I. 701.

Form 9.—Susceptibility of J. W. S. differentiates this form from form 2; Williston Golden appreciably less susceptible than to form 2.

Form 10.—Apparently the least virulent of all forms. Under suboptimum conditions Williston Brown resistant; under optimum conditions semiresistant. Buda, Williston Golden, Akmolinsk, and Kenya slightly more resistant than to form 1; Argentine C. I. 705 much more resistant.

Form 11.—The immunity of the "very pale blue crimped" (C. I. 647) differentiates this form from form 6. This is the only form to which this variety is highly resistant.

Form 12.—Kenya semiresistant, uredia large, well developed, aggregated in the heavily inoculated regions that become chlorotic and tend to dry up prematurely; Buda susceptible; Williston Golden resistant; Akmolinsk highly resistant; J. W. S. nearly immune. Differentiated from form 4 by the more susceptible reaction of Kenya and the less susceptible reaction of Buda.

Form 13.—Susceptibility of J. W. S. differentiates this form from forms 4 and 12.

Form 14.—Buda semiresistant; Williston Golden susceptible; Akmolinsk, J. W. S., and Abyssinian C. I. 701 nearly immune; Argentine C. I. 705 resistant; and Kenya very resistant. Differentiated from form 2 by semiresistance of Buda and the somewhat less susceptibility of Williston Golden.

GEOGRAPHIC DISTRIBUTION

The localities from which the 99 rust collections were obtained are shown in table 2. More than one form was obtained from several of the collections made in 1934, so that a total of 105 form identifications were made. The distribution of these forms, according to years and States or Provinces in which each was collected, is also shown in table 2.

Physiologic forms 1 to 7, inclusive, were differentiated on the reaction of three differential varieties, Buda, Williston Golden, and Akmolinsk. Since only the type specimen of each of these 7 forms was available for tests with the additional differentials used in the 1934 trials, it is possible that some of the earlier form identifications were not exact. For instance, form 9, which is distinguished from form 2 by the reaction of J. W. S., would have been classified as form 2 prior to the use of J. W. S. as a differential in 1934. Likewise, collections having pathogenic properties of form 10 would have been classed as form 1; and collections having the reactions of forms 12 and 13, as form 4.

TABLE 2.—Geographic distribution of physiologic forms of *Melampsora lini* and number of times each form was isolated from *Linum usitatissimum*, 1931-34

Year	State or Province	Place collected ¹	Number of isolates of physiologic form—														Total number
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1931	Manitoba, Canada	St. Adolphe ²	1														1
		Winnipeg ²				2											2
		Total	1			2											3
	North Dakota	Cooperstown															
		Fargo	1			2											3
		Hamilton															
		Langdon	1														1
		Mandan	1	1													2
		New Salem	1														1
		Wishek	1														1
		Total	4	2	1	4											11
	Total		5	2	1	6											14
1932	Minnesota	Cooncreek ¹		2		1											3
		Lamberton	1														1
		Morris				1											1
		Northfield		1													1
		St. Paul			1												1
		Tyler	1														1
		Waseca															1
		Willmar				1											1
		Total	2	3	3	2											10
	North Dakota	Carrington			1												1
		Cavaler	1														1
		Cooperstown			1		1										2
		Grafton		1													1
		Hudsonfield	1														1
		Langdon		1													1
		Lawton					1										1
	South Dakota	Mandan					2										2
		Valley City	1														1
		Total	3	3	2	3	1										12
	Total		5	6	6	5	1										23
		Watertown			1												1
																	1
																	5

¹ Unless stated otherwise, collections were made by H. H. Flor. ² 1 collection was made by F. J. Greaney. ³ 1 collection of physiologic form 2 was made by G. C. Allison.

1934.	Minnesota.	Ada.....	2															2						1
		Alexandria.....	1															1						1
		Averill.....																						1
		Borup.....																						1
		Brandon.....																						1
		Breckenridge.....																						1
		Coon Creek ¹																						1
		Crookston.....	2																					1
		Eldred.....																						1
		Fergus Falls.....																						1
		Northcote ¹																						1
		Owatonna.....																						1
		Waseca.....																						1
		Windom.....	1																					1
		Total.....	6	3	2	2	4	1																12
	North Dakota.	Argusville.....																						1
		Cassation.....																						1
		Fargo.....	2																					1
		Gillsboro.....																						1
		Leonard.....																						1
		Total.....	2	1	1		2																	7
	Oregon.	Corvallis ⁴																						1
	Total.....		8	4	3	2	6	1																13
1931-34.	Manitoba.....		1																					1
	Minnesota.....		9	8	7	4	5	2																3
	North Dakota.....		12	11	5	11	7																	46
	Oregon.....																							1
	South Dakota.....																							1
	Grand total.....		22	19	13	17	12	2	1	1	2	1	1	4	1	1	9	105						14

¹ 1 collection of physiologic form 2 was made by C. C. Allison.⁴ Collected by E. B. Robinson.⁵ Physiologic forms 2 and 3 were collected by C. C. Allison.

A number of physiologic forms of flax rust are widely disseminated throughout the flaxseed-producing area of Minnesota, North Dakota, South Dakota, and Manitoba. Forms 1, 2, and 4 apparently were most prevalent during this 4-year period, but the limited number of collections studied scarcely warrants drawing definite conclusions concerning the relative prevalence of the different forms. Apparently, forms 3 and 5 also were wide-spread, as each was secured from several widely separated localities. Form 6 was obtained from only 2 collections, 1 from Fergus Falls, Minn., in 1933, and the other from Owatonna, Minn., in 1934. Only two collections were obtained outside the flaxseed-producing area. One of these, collected at Astoria, Oreg., yielded form 7 and the other, gathered near Corvallis, Oreg., gave form 8. These two forms are very distinct from all those collected in the flaxseed area and also from each other. In 1934, forms 10, 11, and 13 were each obtained but once; form 9, twice; form 12, 4 times; and form 14, 9 times. Forms 12 and 14 were found in widely scattered localities in Minnesota and North Dakota. The latter form was obtained from more collections in 1934 than was any other.

Several forms have been obtained from the same locality, although the number of collections made in each has been rather limited. Forms 1, 2, 4, 12, and 14 have been obtained from 11 collections made near Fargo, N. Dak.; forms 1, 2, 3, and 4, from 5 collections made near Langdon, N. Dak.; forms 2, 4, 5, and 11, from 5 collections made at Cooncreek (near Anoka), Minn.; and forms 2 and 4, from 3 collections made at Mandan, N. Dak.

HOST RANGE AND VARIETAL RESISTANCE

The results of inoculating 165 varieties of flax with physiologic forms 1 to 5, inclusive, are given in table 3. As only 13 of the 165 varieties tested gave indications of being rust differentials, the reaction of each variety to each form has not been tabulated separately. The varieties have been grouped according to the flax type to which they belong. The percentages of plants of each variety that were immune, resistant, or susceptible are given, as is also the general rust reaction of each variety.

TABLE 3.—Reaction of varieties of *Linum usitatissimum* to five forms of *Melampsora lini*

Group and variety	C. I. no.	Im- mune	Resist- ant	Suscep- tible	General rust re- action ¹
<i>Linum usitatissimum creplians</i> (dehiscent flax):		Percent	Percent	Percent	
From Siberia.....	295	0	0	100	S
From Germany.....	496	0	0	100	S
From Ukraine.....	506	0	0	100	S
Do.....	507	0	0	100	S
<i>L. usitatissimum</i> (seed flax):					
Petals, broad, flat:					
Abyssinian:					
F. P. I. 37086.....	26	0	0	100	S
F. P. I. 58762.....	300	0	0	100	S
F. P. I. 58764.....	302	3	9	88	S
From Egypt.....	380	12	9	79	S
From Fergana, S. E. Turkistan.....	511	0	56	44	D
F. P. I. 60539.....	701	48	10	42	D
From Kenya, East Africa.....	707	0	0	100	S

¹ Letters indicate the various types of rust reaction, as follows: D, differential variety (not reacting similarly to all forms); I, immune (75 percent or more of the plants immune); M, mixed (less than 75 percent of the plants falling into any 1 class); R, resistant (75 percent or more of the plants resistant); S, susceptible (75 percent or more of the plants susceptible).

² F. P. I. refers to accession number of the Division of Plant Exploration and Introduction.

TABLE 3.—Reaction of varieties of *Linum usitatissimum* to five forms of *Melampsora lini*—Continued

Group and variety	C. I. no.	Im- mune	Resist- ant	Suscep- tible	General rust re- action
<i>L. usitatissimum</i> (seed flax)—Continued.					
Petals, broad, flat—Continued.					
American:					
Blue-flowered:					
Bison.....	389	0	0	100	S
Blanc.....	323-c	0	0	100	S
Bolley No. 1823.....	754	0	0	91	S
Buda.....	270	0	54	46	S
Do.....	326	0	0	100	D
Diadem.....	321	2	31	67	D
Linota.....	244	0	0	100	S
Minnesota 24-410.....	421	0	0	100	S
Minnesota 25-202.....	447	0	0	100	S
Minnesota 25-221.....	423	0	0	100	S
Minnesota 25-245.....	446	0	0	100	S
Minnesota 281.....	438	0	0	100	S
North Dakota Resistant 5.....	411	0	0	100	S
North Dakota Resistant 52.....	275	0	0	100	S
North Dakota Resistant 114.....	489	0	0	100	S
North Dakota Resistant 714.....	399	0	0	100	S
North Dakota Resistant 726.....	412	0	3	97	S
North Dakota 40016.....	428	0	0	100	S
Pale blue.....	387-1	0	0	100	S
Redwing.....	320	0	0	100	S
Do.....	458	0	2	98	S
Do.....	499	0	51	49	D
Slope.....	274	0	0	100	S
Walsh.....	645	85	15	0	I
Winona.....	481	0	0	100	S
White-flowered:					
Ottawa white-flowered..	24	0	0	100	S
Pink-flowered:					
Bolley Golden.....	644	70	30	0	M
Bolley No. 1821.....	751	100	0	0	I
Bolley No. 1822.....	750	100	0	0	I
Lethbridge Golden.....	23	5	5	90	S
Long No. 4.....	400	0	0	100	S
Long No. 66.....	337	0	0	100	S
Do.....	719	0	0	100	S
Long No. 83.....	354	0	0	100	S
Pale pink.....	173-1	0	100	0	R
Do.....	173-3	0	100	0	R
Do.....	649	0	100	0	R
Williston Golden.....	25	0	56	44	D
Argentine:					
North Dakota 1742.....	342	97	0	3	I
Minnesota 25-343.....	417	90	1	9	I
Minnesota 25-341.....	462	100	0	0	I
Minnesota 25-362.....	472	100	0	0	I
Minnesota 25-330.....	690	100	0	0	I
Minnesota 25-361-1.....	691	100	0	0	I
Minnesota 27-379-3.....	692	100	0	0	I
Minnesota 25-323.....	705	88	12	0	D
Biglow.....	414	97	0	3	I
Cape.....	720	21	53	26	M
Do.....	721	100	0	0	I
Do.....	722	94	6	0	I
Kenya.....	708	96	4	0	I
Do.....	709	80	18	2	D
Light mauve.....	379-1	24	70	0	R
Lino Grande.....	381-2	52	45	3	M
Long No. 8.....	466	94	6	0	I
Malabrigo.....	346	96	4	0	I
Do.....	666	100	0	0	I
Rio.....	280	100	0	0	I
Rosario.....	316	3	97	0	R
Indian:					
Howard and Khan (13):					
var. <i>luteum</i> , type 1.....		0	0	100	S
var. <i>cyaneum</i> , type 8.....		0	0	100	S
var. <i>purpureum</i> , type 11.....		5	0	95	S
var. <i>album</i> , type 12.....		34	0	66	M
var. <i>album</i> , type 15.....		0	0	100	S
var. <i>agreste</i> , type 22.....		0	0	100	S
var. <i>meridionale</i> , type 23.....		0	0	100	S
var. <i>prolense</i> , type 25.....		0	0	100	S
var. <i>minor</i> , type 29.....		100	0	0	I
var. <i>pulchrum</i> , type 34.....		100	0	0	I
var. <i>commune</i> , type 46.....		100	0	0	I
var. <i>commune</i> , type 48.....		100	0	0	I

TABLE 3.—Reaction of varieties of *Linum usitatissimum* to five forms of *Melampsora lini*—Continued

Group and variety	C. I. no.	Im- mune	Resist- ant	Suscep- tible	General rust re- action
<i>L. usitatissimum</i> (seed flax)—Continued.					
Petals, broad, flat—Continued.					
Indian—Continued.					
Howard and Kahn (13)—Continued.					
var. <i>commune</i> , type 53.....		Percent 90	Percent 0	Percent 1	I
var. <i>commune</i> , type 55.....		0	99	1	R
var. <i>campestre</i> , type 68.....		99	1	0	I
var. <i>sativum</i> , type 121.....		41	59	0	M
Shaw, Khan, and Alam (19), type 124.....	710	29	71	0	M
Mediterranean:					
Beladi.....	377	75	25	0	I
Creta.....	31-1	57	28	15	M
Cyprus.....	683	55	4	41	M
Giza.....	378	100	0	0	I
Morocco.....	376-2	96	0	4	I
Russian:					
Akmolinsk.....	515	11	15	74	D
Do.....	520	17	3	20	D
Billings.....	184	2	2	96	S
Crimean.....	563	27	5	68	M
Damont.....	3	99	0	1	I
Fergana.....	512	0	0	100	S
Newland.....	188	96	3	1	I
North Caucasian.....	620	45	13	42	M
Novelty.....	140	0	19	81	S
Pale blue.....	176	5	95	0	R
Samarkand.....	514	5	16	79	D
Winter:					
Roman winter.....	470	40	8	52	M
Hybrid:					
Reserve X Morye.....	486	19	25	56	M
Saginaw X Ottawa 770 B.....	675	100	0	0	I
Do.....	676	100	0	0	I
Do.....	677	100	0	0	I
Winona X Ottawa 770 B.....	651	100	0	0	I
Do.....	652	100	0	0	I
Do.....	653	100	0	0	I
Do.....	654	92	0	8	I
Do.....	656	100	0	0	I
Do.....	657	100	0	0	I
Do.....	658	98	0	2	I
Do.....	660	3	0	97	S
Do.....	661	97	1	2	I
Do.....	662	100	0	0	I
Do.....	664	100	0	0	I
Do.....	667	100	0	0	I
Do.....	671	100	0	0	I
Do.....	672	100	0	0	I
Do.....	673	100	0	0	I
Do.....	674	100	0	0	I
Do.....	681	100	0	0	I
Do.....	682	100	0	0	I
Do.....	697	100	0	0	I
Do.....	711	2	34	64	M
Do.....	716	100	0	0	I
La Plata.....	324	91	5	4	I
Long No. 125.....	356	40	0	60	M
North Dakota 40046.....	492	93	4	3	I
Tammes light blue.....	332	100	0	0	I
Tammes pale blue.....	333	97	0	3	I
Petals, narrow, margins crimped ³					
Ecu-olive 4 seed.....	325	0	0	100	S
Minnesota 25-125.....	392	0	0	100	S
Minnesota 29-39.....	664	100	0	0	I
Minnesota 29-55.....	685	24	0	76	S
Minnesota 29-76.....	686	100	0	0	I
Minnesota 29-45.....	687	100	0	0	I
Ottawa 770 B.....	355	100	0	0	I
Ottawa 829 C.....	391	0	0	100	S
Pale blue.....	646	4	9	87	S
Tammes crimped.....	330	0	0	100	S
"Very pale blue crimped".....	647	5	31	64	D

³ The word "crimped" is here used to describe the petal margins, which are incurved and somewhat wavy.

⁴ RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

TABLE 3.—Reaction of varieties of *Linum usitatissimum* to five forms of *Melampsora lini*—Continued

Group and variety	C. I. no.	Im- mune	Resist- ant	Suscep- tible	General rust re- action.
<i>L. usitatissimum</i> (fiber flax):					
Petals, narrow, margins crimped—Continued.					
Blue-flowered:					
Althausen.....	628	0	0	100	S
Do.....	630	7	0	93	S
Dalgonetz.....	498	0	0	100	S
F. I. No. 3.....	694	2	6	92	S
J. W. S., ¹ F. P. I. 73560.....	383	3	8	89	S
J. W. S.....	708	16	0	84	D
Peerless.....	895	1	0	99	S
Saginaw.....	449	7	3	90	S
White-flowered:					
Friesland white.....	56-1	0	0	100	S
Minnesota 25-64.....	420	0	0	100	S
Pinnacle.....	693	0	2	98	S
Saginaw white.....	448	0	0	100	S
Tammes white.....	329	0	0	100	S
Tammes yellow seed.....	331	0	0	100	S
Pink-flowered:					
Deep pink.....	648	0	0	100	S
Pale pink.....	479	0	0	100	S
Tall pink.....	451-3	0	1	99	S
Do.....	451-3	0	0	100	S
Tammes deep pink.....	334	0	0	100	S
Do.....	336	0	0	100	S

¹ J. W. S. are the initials of J. W. Stewart, the originator of the variety.

When 75 percent or more of the tested plants in a variety fell into a single reaction class, the variety, if not differential, was thought to possess the reaction of that class; when less than 75 percent of the tested plants fell into a single reaction class, the variety was considered as too heterogeneous to be classified and was termed "mixed." Approximately twelve 30-day-old plants of each variety were inoculated with each rust form, and in cases where the classification was in doubt the tests were repeated. The percentage figures, therefore, are based on the reaction of 60 or more plants.

The lack of genetic purity of the varieties for rust reaction was one of the striking features of this test. Thirty-nine varieties were pure for rust immunity, but twenty-two of these were Minnesota hybrids that had been selected particularly for this quality. Twenty-two additional varieties had from 75 to 99 percent of the plants immune. Twenty of these were classed as immune and two as differentials. In a majority of the varieties included in this group most of the plants lacking immunity were highly resistant. Fifty-three varieties were pure for susceptibility. An additional 22 had from 75 to 99 percent of their plants susceptible. Nineteen of these were classed as susceptible and three as differentials. Seven varieties were classed as resistant, 3 of which were pure for resistance and 3 showed plants either resistant or immune. Fourteen varieties were so heterogeneous that they could not be classified as to predominance of reaction, and were classed as mixed. Additional differentials might have been obtained from this last group, but their reactions to the five forms did not indicate them to be such. Thirteen varieties were found to contain rust-differentiating strains, and were classed as differential varieties.

The varieties and strains included in this test do not exhaust the possibilities for obtaining immune, resistant, or susceptible lines in any of the groups. These varieties were chosen because they were

typical of the different groups and of diverse geographical origin or because they possessed commercial possibilities, and they probably are representative of their respective groups in regard to rust reaction. The dehiscent flaxes, *Linum usitatissimum* var. *crepitans*, were pure for rust susceptibility. This was the only major group in which all varieties and strains were consistently pure for one type of rust reaction. The American blue-flowered and white-flowered seed flaxes and the white-flowered and pink-flowered fiber flaxes were predominantly susceptible, but some varieties in both of these groups possessed plants that were either resistant or immune. The Abyssinian varieties and the blue-flowered fiber flaxes also were predominantly susceptible, but most of the varieties in these groups were mixtures in which some plants were either resistant or immune. The Saginaw \times Ottawa 770 B hybrids were pure for immunity. The Winona \times Ottawa 770 B hybrids and the Mediterranean and Argentine varieties were predominantly immune, but a number of varieties in these groups possessed plants the reactions of which ranged from complete immunity to extreme susceptibility. The other hybrids and the Russian varieties were heterogeneous in reaction, most varieties in these two groups having plants falling into all three rust-reaction categories. Varieties with petal margins crimped were relatively pure for rust reaction.

STABILITY OF PHYSIOLOGIC FORMS

Physiologic forms 1 to 4 of *Melampsora lini* have been in culture for approximately 4 years and have passed through 20 to 30 urediospore generations. In none of these forms nor in any of the others that have been studied has there been any indication of a change in pathogenicity.

DISCUSSION

The effect of the discovery of physiologic forms of *Melampsora lini* on measures to control this rust through the development and use of resistant varieties is, of course, problematical. Thus far, 14 physiologic forms have been distinguished by the reaction of seven differential hosts. It seems probable that more forms will be isolated when additional differentials are discovered and when more rust collections are studied. Allen (1) has shown that *M. lini* is heterothallic. The importance of this phenomenon in the origin of new forms of flax rust is not yet known. However, it has been demonstrated (16, 22) that the aecial stage on the barberry is a fertile field for the development of new physiologic forms of *Puccinia graminis tritici* Eriks. and Henn. Since the process of aecial formation in the two rusts is comparable, there is no reason to believe that new forms of *M. lini* may not originate in the aecial stage on flax. The destruction of the common barberry, the aecial host of *P. graminis* Pers., would effectively prevent the production of new forms of *P. graminis* by hybridization. *M. lini*, however, is a eu-autoecious rust, and therefore to preclude the opportunity for the development of new rust forms through hybridization would necessitate the complete destruction of all rusted portions of flax plants from previous crops, an impracticable task.

Although *Melampsora lini* appears to be highly specialized, not only on various other species of *Linum* (5, 8, 9, 17), but, as has been demonstrated by the present tests, on *L. usitatissimum*, and although it possesses abundant opportunities for hybridization and the subsequent development of new forms, the prospects of rust control through the development of resistant varieties appear to be bright. The 165 varieties of flax showed surprisingly little specific response to five forms of flax rust. This is the more remarkable when it is considered that these 165 varieties represented the fruit of a systematic effort to obtain diverse types of *L. usitatissimum* from all parts of the world. Of the 165 varieties, 152 gave no indication of possessing rust-differentiating potentialities. Sixty-one varieties were predominantly immune from all five forms with which they were tested. Since these immune varieties comprised a number of flax types, and since Henry (10) has shown that immunity from flax rust is transferred independently of wilt resistance or morphologic type, there should be no lack of immune breeding material.

The breeding program, already in progress, for the development of rust-resistant varieties of flax, appears to rest on a sound basis. Henry (10) found Ottawa 770 B and certain strains of Williston Golden and Argentine to be immune from rust. In the present investigations, certain forms of rust were found to attack all strains of Williston Golden, but Ottawa 770 B and certain Argentine selections have been consistently immune. Henry used only the latter two as rust immune parents in his breeding work, and most of the Minnesota selections that have been derived from these crosses were immune from the five forms of rust with which they were tested. Thus far, there has been no evidence of the existence of a form of flax rust capable of attacking Ottawa 770 B, certain Argentine selections or varieties possessing similar factors for resistance. Nevertheless, it would be desirable to subject these immune varieties to rust collections from other parts of the world to determine whether forms capable of attacking them exist. Stakman, Levine, and Bailey (21) found White Tartar oats resistant to all forms of *Puccinia graminis avenae* Eriks. and Henn. collected in North America, but susceptible to forms of this rust obtained from Sweden and South Africa. If forms of *Melampsora lini* capable of attacking the immune flax varieties of the United States exist in other parts of the world, measures should be taken to assure their exclusion from this country, since it has been demonstrated that teliospores on bits of straw and chaff that are often present with the seed are carriers of flax rust (10).

The heterogeneous rust reaction of so many of the varieties was surprising. Apparently natural hybridization plays a more important role than has sometimes been supposed (4) or else the nursery technique is at fault. It appears more probable that the former is the case. Henry and Tu (12) found, under conditions at St. Paul, Minn., that natural crossing between varieties in adjacent rows or even in rows several feet apart was too important a factor to be neglected. Apparently, this condition prevails in other localities, since 70 of the 165 varieties studied gave heterogeneous rust reaction. Bagging of individual plants and isolation of the separate varieties in the field may be necessary in order to insure the maintenance of varietal purity. It is possible that a test to determine rust reaction, made in conjunction with the certification of rust-resistant varieties, would aid materially in maintaining varietal purity.

SUMMARY

Strains of Buda (C. I. 270), Williston Golden (C. I. 25), Williston Brown (C. I. 803), Akmolinsk (C. I. 515), J. W. S. (C. I. 708), Kenya (C. I. 709), "very pale blue crimped" (C. I. 647), Argentine (C. I. 705), and Abyssinian (C. I. 701) have been selected as differential varieties for the identification of physiologic forms of *Melampsora lini* (Pers.) Lév. on *Linum usitatissimum* L.

From 99 collections of *Melampsora lini* made in the United States and Canada, 14 physiologic forms have been distinguished by the reaction of the differential varieties named above.

Several forms of the rust were found to be widely distributed in Minnesota, North Dakota, South Dakota, Oregon, and Manitoba. In several localities more than one form was found.

In the 4-year period covered by these investigations four rust forms have passed through 20 to 30 urediospore generations without any apparent change in pathogenicity.

Pathogenicity tests indicated that as a rule flax varieties are not specific in their reaction to the different forms of rust. Of 165 varieties tested, only 13 gave differential reactions to five forms.

Pathogenicity tests showed that many varieties were not pure lines in respect to rust reaction. Lines pure for this characteristic have been readily obtained by a process of plant selection.

LITERATURE CITED

- (1) ALLEN, R. F.
1933. THE SPERMATIA OF FLAX RUST, MELAMPSORA LINI. (Phytopathological note) Phytopathology 23: 487.
- (2) ARTHUR, J. C.
1907. CULTURES OF UREDINEAE IN 1906. Jour. Mycol. 13: 189-205.
- (3) BAILEY, D. L.
1925. PHYSIOLOGIC SPECIALIZATION IN PUCCINIA GRAMINIS AVENAE ERIKSS. AND HENN. Minn. Agr. Expt. Sta. Tech. Bull. 35, 33 pp., illus.
- (4) BOLLEY, H. L.
1927. INDICATIONS OF THE TRANSMISSION OF AN ACQUIRED CHARACTER IN FLAX. Science (n. s.) 66: 301-302.
- (5) BUCHHEIM, A.
1915. ZUR BIOLOGIE VON MELAMPSORA LINI. Ber. Deut. Bot. Gesell. 33: 73-75.
- (6) COTTER, R. U., and LEVINE, M. N.
1932. PHYSIOLOGIC SPECIALIZATION IN PUCCINIA GRAMINIS SECALIS. Jour. Agr. Research 45: 297-315, illus.
- (7) DORST, J. C.
1923. RESISTANCE OF SEVERAL STRAINS OF WHITE FLOWERING FLAX TO MELAMPSORA LINI. Internatl. Conf. Phytopath. and Econ. Ent., p. 33. Wageningen and Baarn. Holland.
- (8) FUECKEL, L.
1869-70. SYMBOLAE MYCOLOGICAE, BEITRÄGE ZUR KENNTNISS DER RHEINISCHEN PILZE. 459 pp., Wiesbaden. (Nassauisch. Ver. Naturk. Jahrb. 23-24, 1869-70).
- (9) HART, H.
1926. FACTORS AFFECTING THE DEVELOPMENT OF FLAX RUST, MELAMPSORA LINI (PERS.) LÉV. Phytopathology 16: 185-205, illus.
- (10) HENRY, A. W.
1926. FLAX RUST AND ITS CONTROL. Minn. Agr. Expt. Sta. Tech. Bull. 36, 20 pp., illus.
- (11) ———
1928. REACTION OF LINUM SPECIES OF VARIOUS CHROMOSOME NUMBERS TO RUST AND POWDERY MILDEW. (Abstract) Sci. Agr. 8: 460-461.
- (12) ——— and TU, C.
1928. NATURAL CROSSING IN FLAX. Jour. Amer. Soc. Agron. 20: 1183-1192.

- (13) HOWARD, G. L. C., and KHAN, A. R.
1924. STUDIES IN INDIAN OIL SEEDS. NO. 2. LINSEED. India Dept. Agr. Mem., Bot. Ser. 12: 135-183, illus.
- (14) LAFFERTY, H. A., RHYNEHART, J. G., and PETHYBRIDGE, G. H.
1922. INVESTIGATIONS ON FLAX DISEASES. (THIRD REPORT.) Ireland Dept. Agr. and Tech. Instr. Jour. 22: 103-120, illus.
- (15) LÉVEILLÉ, J. H.
1847. SUR LA DISPOSITION MÉTHODIQUE DES URÉDINÉES. Ann. Sci. Nat., Bot. (3) 8: 369-376.
- (16) NEWTON, M., JOHNSON, T., and BROWN, A. M.
1930. A PRELIMINARY STUDY ON THE HYBRIDIZATION OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI. Sci. Agr. 10: 721-731, illus.
- (17) PALM, B.
1910. NYA BIDRAG TILL STOCKHOLMSTRAKTENS SVAMPFLORA. Svensk Bot. Tidskr. 4: (1)-(8).
- (18) PERSOON, C. H.
1801. SYNOPSIS METHODICA FUNGORUM. SISTENS ENUMERATIONEM OMNIUM HUC USQUE DETECTARUM SPECIERUM, EUM BREVIBUS DESCRIPTIONIBUS NEC NON SYNONYMIS ET OBSERVATIONIBUS SELECTIS. 2 v., illus. Gottingae.
- (19) SHAW, F. J. F., KHAN, K. A. R., and ALAM, M.
1931. STUDIES IN INDIAN OIL SEEDS. V. THE INHERITANCE OF CHARACTERS IN INDIAN LINSEED. Indian Jour. Agr. Sci. 1: 1-57, illus.
- (20) STAKMAN, E. C., and LEVINE, M. N.
1922. THE DETERMINATION OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON TRITICUM SPP. Minn. Agr. Expt. Sta. Tech. Bull. 8, 10 pp., illus.
- (21) ——— LEVINE, M. N., and BAILEY, D. L.
1923. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON VARIETIES OF AVENA SPP. Jour. Agr. Research 24: 1013-1018, illus.
- (22) ——— LEVINE, M. N., and COTTER, R. U.
1930. ORIGIN OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS THROUGH HYBRIDIZATION AND MUTATION. Sci. Agr. 10: 707-720.
- (23) TOBLER, F.
1921. ZUR KENNTNIS DER LEBENS- UND WIRKUNGSWEISE DES FLACHEROSTES. Faserforschung 1: 223-229, illus.

INTRACAPSULARY BOLLS IN ASIATIC COTTON¹

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INTRODUCTION

The presence of an abnormal growth in cotton bolls, which was interpreted as a formation of supernumerary carpels, was reported by Meade² in 1913 and described as follows:

In its simplest form the abnormality consisted of a solid, elongated, whitish body, developed in the center of the boll between the placentae. In many instances, however, this body was divided into from two to five longitudinal compartments, resembling miniature locks, which inclosed rudimentary ovules. In some cases it extended nearly the entire length of the boll, but was always unattached except at the base.

One upland cotton boll was observed by Meade at Clarksville, Tex., in which this abnormal growth was sufficiently developed to form a small rudimentary boll containing two apparently mature seeds.³

At the United States Acclimatization Garden, Bard, Calif., in 1932 and 1933, numerous rudimentary bolls were observed in the bolls of a strain of *Gossypium herbaceum* L. introduced from Tashkent, Uzbek, Union of Soviet Socialist Republics. These rudimentary bolls are described in the present paper. No observations were made of the bolls produced by this strain of cotton prior to 1932, but simple forms of the abnormal growth were present in a few of the bolls saved for herbarium specimens during 1930 and 1931. Simple forms of this abnormality were also observed in the bolls of two other strains of *G. herbaceum* and in a few bolls of *G. davidsoni* Kellogg grown in the same field in 1932. Numerous bolls were examined in 1932 and 1933 of other strains of *G. herbaceum*, *G. hirsutum* L., *G. barbadense* L., *G. nanking* Meyen, *G. neglectum* Tod., and several other species of cotton, but no indication of this abnormal growth was observed. However, simple forms of this abnormality have been observed frequently in varieties of upland cotton in other localities and in a few bolls of *G. barbadense* at Glendale, Calif.²

The term "supernumerary carpels" does not adequately describe this abnormal growth when it reaches such a stage of development that small rudimentary bolls are formed. Therefore, in this paper the term "supernumerary carpels" has been applied to the simple forms of this growth (fig. 1, A), and the term "intracapsulary bolls" has been used in cases where the growth was sufficiently developed to form small rudimentary bolls (fig. 1, C).

¹ Received for publication July 1, 1935; issued January 1936.

² MEADE, R. N. SUPERNUMERARY CARPELS IN COTTON BOLLS. U. S. Dept. Agr., Bur. Plant Indus. Circ. 111: 25-28, illus. 1913.

³ A study of supernumerary carpels, in relation to the general morphology of the cotton boll, has been made by W. W. Ballard, of the Bureau of Plant Industry. (Unpublished data.)

DESCRIPTION OF INTRACAPSULARY BOLLS

Fourteen intracapsulary bolls were found on the five plants of *Gossypium herbaceum* growing at Bard, Calif., in 1932, and all closely resembled in color and structure the normal bolls in which they were produced. The outer tissue was rather thin but was dotted with numerous oil glands. Some of the intracapsulary bolls were of normal shape, but others were somewhat lop-sided from being pressed against the placentae of the bolls in which they were produced. They appeared, from the outer surface, to be divided into 2, 3, or 4 carpels, but when broken were found to consist of only a single carpel containing from 1 to 3 mature seeds. The placentae were not sufficiently developed to form separate carpels, and the seeds were attached near the base of the bolls. In a few cases a very slender structure was observed to extend upward from the apex of the intracapsulary bolls; this probably had the function of a style and conducted the pollen to the ovules.

The seeds, which were slightly rounder and in some instances larger than those produced in the normal bolls, were covered with the usual coating of short fuzz, and all but two had lint that ranged from 12 to 24 mm in length. Ten of the nineteen seeds that developed in the intracapsulary bolls germinated when planted in 1933 and produced normal healthy plants. In addition to the seeds, a number of rudimentary ovules were developed in the intracapsulary bolls and supernumerary carpels.

The size of bolls, number of seeds per boll, number of boll divisions, and the length and quality of lint in the intracapsulary bolls and in normal bolls are given in table 1. Figure 1 shows the various stages of development in the bolls.

TABLE 1.—Size of bolls, number of seeds per boll, number of boll divisions, and length and quality of lint in intracapsulary bolls and in normal bolls of *Gossypium herbaceum* at Bard, Calif., 1932

Boll no.	Size of bolls		Seeds per boll	Boll divisions	Length of lint	Quality of lint
	Diameter	Length				
	Mm	Mm	Number	Number	Mm	
1.....	6	9	1	2	No lint.
2.....	8	10	1	2	Do.
3.....	10	9	1	3	23	Fairly strong.
4.....	9	11	1	2	22	Very weak.
5.....	12	11	2	4	16	Very weak and sparse.
6.....	7	10	1	3	17	Do.
7.....	14	17	3	4	15	Do.
8.....	8	10	1	3	17	Do.
9.....	7	11	1	2	20	Do.
10.....	13	15	2	4	19	Fairly strong.
11.....	11	13	1	3	24	Very weak and sparse.
12.....	11	14	2	4	19	Do.
13.....	12	12	1	4	18	Do.
14.....	8	10	1	3	20	Fairly strong.
Mean for normal bolls.....	27	25	18-30	3-5	21	Do.
					17	Do.
					12	Very weak and sparse.
					25	Generally soft and not very strong.

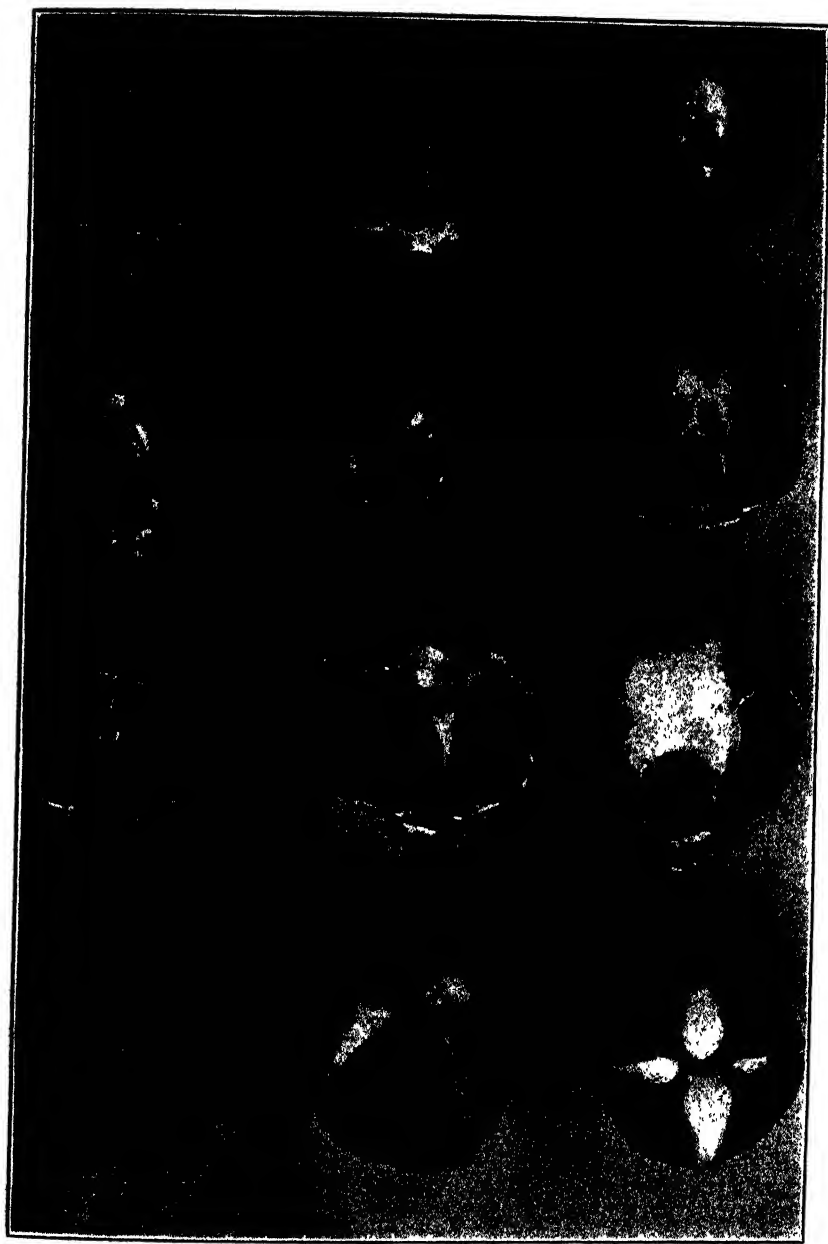


FIGURE 1.—Bolls of *Gossypium herbaceum* showing various stages of development of supernumerary carpels from (A) the simplest form to (C) the intracapsulary bolls. Two normal bolls of this strain are shown at the right in D.

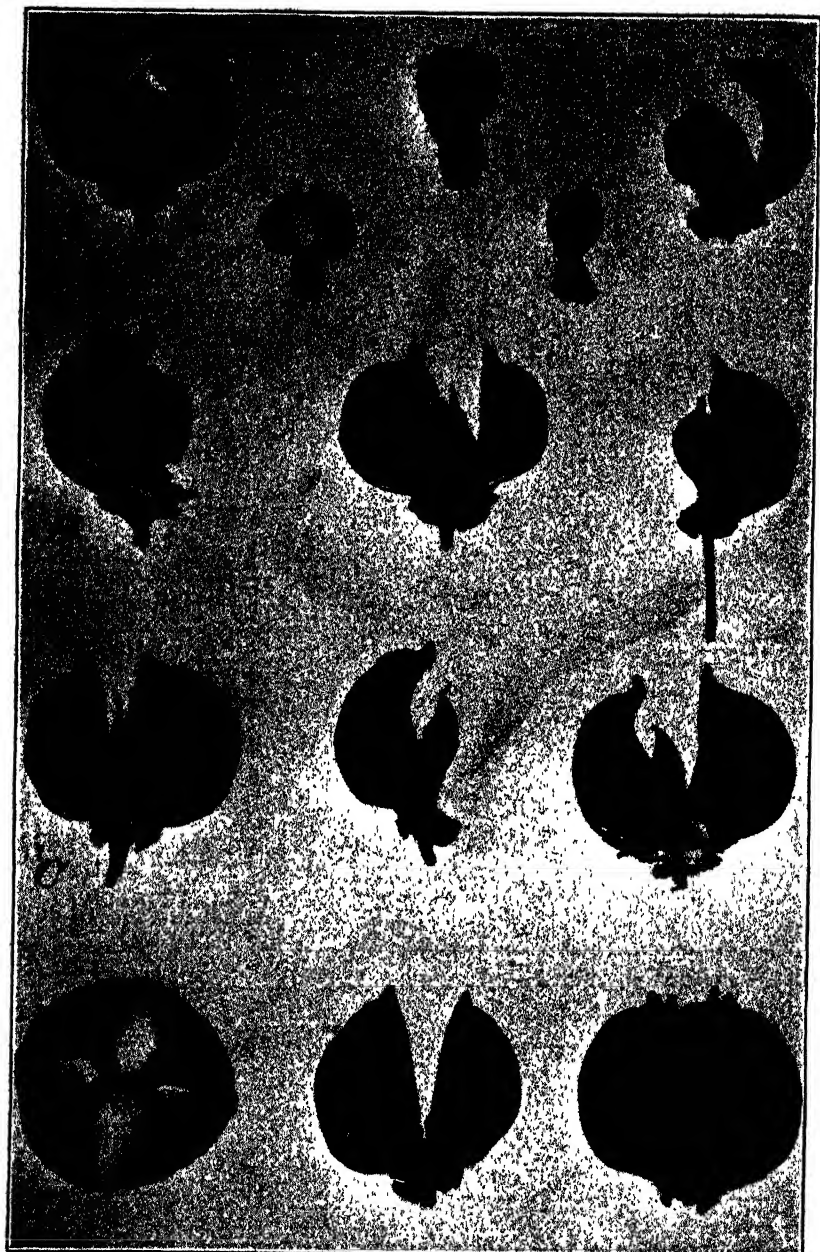


FIGURE 2.—Bolls of *Gossypium herbaceum* with seed cotton removed, showing (A) detached, and (B) attached, intracapsulary bolls, (C) supernumerary carpels, and (D) normal bolls.

Figure 2 shows bolls with seed cotton removed. As viewed from outside, 4 of the intracapsulary bolls appeared to be divided into 2 carpels, 5 into 3 carpels, and 5 into 4 carpels. Ten bolls contained 1 mature seed each, 3 contained 2 seeds each, and 1 had 3 seeds. On the 17 seeds that produced lint, the shortest lint was 12 mm and the longest 24 mm. The smallest intracapsulary boll was 6 mm in diameter and 9 mm in length, and the largest was 14 mm in diameter and 17 mm in length. The normal bolls of this strain have from 3 to 5 carpels and produce 18 to 30 seeds with lint averaging 25 mm in length. The normal bolls average 27 mm in diameter and 25 mm in length. It will be noted from these measurements that the normal bolls are broader than long, whereas 11 of the 14 intracapsulary bolls were longer than they were broad. This was probably due to the fact that the intracapsulary bolls could develop more easily along the placentae of the bolls that contained them than they could expand against the placentae. The placentae in some of the bolls were pressed back, forming a crescent or half circle, by the development of the intracapsulary bolls.

OBSERVATIONS IN 1933

Observations were made in 1933 with the strain of *Gossypium herbaceum* from Tashkent to ascertain (1) what effect seasonal changes have on the development of supernumerary carpels and intracapsulary bolls, (2) whether the frequency of supernumerary carpels and intracapsulary bolls changes as acclimatization proceeds, and (3) whether the abnormal growth character is hereditary. For these observations the 1932 plants were ratooned in the spring of 1933, and additional plantings were made on April 13 with original seed from Tashkent and with seed acclimatized 1, 2, and 3 years at Bard. A planting with seed acclimatized 2 years at Bard was also made at the United States Acclimatization Field Station, State College, N. Mex.⁴

The seed acclimatized 1 year was produced during 1930, and the seed acclimatized 2 years was produced during 1931 from the 1930 seed grown at Bard. The planting made in 1933 with these two seed stocks was from random samples. The seed acclimatized 3 years was produced in 1932 from the seed grown at Bard in 1930 and 1931, and the seed used in the 1933 planting was from the intracapsulary bolls produced in 1932.

EFFECTS OF SEASONAL CHANGES AND ACCLIMATIZATION

The seed planted April 13, 1933, germinated readily and the seedlings made rapid growth. The plants began flowering in June and continued until October. The first bolls matured in August and the last in November. The 1932 plants were ratooned in March 1933 and by May had begun flowering, which continued until October. The first bolls on these plants matured in July and the last in November.

In order to determine the effect of seasonal changes on the presence and development of the abnormal growth character, the period during which the bolls matured was divided into four 30-day periods. During the first period, July 4 to August 3, the ratooned plants matured 261 bolls, of which 50, or 19.15 percent, contained supernumerary carpels. No bolls were matured during this period by the

⁴ Planting was made and data were collected by A. R. Leding, chief scientific aid.

plants of the 1933 planting. During the second period, August 4 to September 3, supernumerary carpels were present in 36 percent of the bolls matured on the ratooned plants and in 42.1, 42.7, 42.2, and 41.2 percent of the bolls matured on the plants grown in 1933 from the original imported seed and from seed representing 1, 2, and 3 years of acclimatization, respectively. Not only did the percentages of bolls containing supernumerary carpels increase during the third and fourth periods, from September 4 to October 4 and from October 5 to November 4, but the abnormal growth was considerably more developed, a total of 130 intracapsular bolls being produced. These data (table 2) were recorded collectively for all plants of each seed stock, but no plants were observed that did not produce a fairly high percentage of such bolls.

TABLE 2.—*Effects of seasonal changes and acclimatization on number of bolls containing supernumerary carpels and intracapsular bolls at Bard, Calif., 1933*

Seed stock	Year planted	Plants	Period of maturity	Bolls			Bolls containing supernumerary carpels and intracapsular bolls
				Total	With supernumerary carpels	Intracapsular	
		Number		Number	Number	Number	Percent
Original seed imported from Union of Soviet Socialist Republics.	1932	5	July 4-Aug. 3.....	261	50	0	19.15
			Aug. 4-Sept. 3.....	211	76	0	36.0
			Sept. 4-Oct. 4.....	188	131	10	75.0
			Oct. 5-Nov. 4.....	86	69	9	90.6
Do.....	1933	3	Aug. 4-Sept. 3.....	76	32	0	42.1
			Sept. 4-Oct. 4.....	125	90	8	78.4
			Oct. 5-Nov. 4.....	107	81	13	87.9
			Aug. 4-Sept. 3.....	178	74	0	42.7
1-year acclimatized seed.....	1933	9	Sept. 4-Oct. 4.....	265	205	15	83.1
			Oct. 5-Nov. 4.....	422	323	41	86.3
2-year acclimatized seed.....	1933	6	Aug. 4-Sept. 3.....	64	27	0	42.2
			Sept. 4-Oct. 4.....	84	66	5	84.5
3-year acclimatized seed.....	1933	6	Oct. 5-Nov. 4.....	99	76	11	87.9
			Aug. 4-Sept. 3.....	83	36	0	41.2
			Sept. 4-Oct. 4.....	71	53	5	81.7
			Oct. 5-Nov. 4.....	189	148	13	85.2

¹ The 1932 plants were ratooned in the spring of 1933.

The increase in the number of supernumerary carpels as the season advanced and the development of the extreme intracapsular boll stage during the latter part of the season indicate that differences in conditions during the season influence this abnormal development.

Data collected on the seven plants observed at State College, N. Mex., in 1933 showed that of a total of 340 bolls which matured 172 contained supernumerary carpels and 3 contained intracapsular bolls with one mature seed each. The percentage of bolls containing supernumerary carpels and intracapsular bolls for these seven plants varied from 28.5 to 65.8 percent, with a mean of 51.5 percent.

No difference was noted in the degree of development of the supernumerary carpels and intracapsular bolls produced by the plants grown from the original seed stock and from seed representing 1, 2, and 3 years acclimatization at Bard, nor were there any significant differences in the percentages of bolls that contained this abnormal growth.

The number of intracapsulary bolls having 2, 3, or 4 boll divisions and the number having 1, 2, or 3 mature seeds are given in table 3.

TABLE 3.—Number of intracapsulary bolls with 2, 3, or 4 divisions and number of these bolls containing 1, 2, or 3 mature seeds, Bard, Calif., 1933

Seed stock	Year of planting	Intracapsulary bolls with indicated number of—					
		Boll divisions			Seeds per boll		
		2	3	4	1	2	3
Original seed imported from Union of Soviet Socialist Republics.....	¹ 1932	Number 3	Number 13	Number 3	Number 15	Number 4	Number 0
Do.....	1933	6	13	2	19	2	0
1-year acclimatized seed.....	1933	14	38	4	49	6	1
2-year acclimatized seed.....	1933	3	12	1	14	2	0
3-year acclimatized seed.....	1933	4	11	3	14	3	1

¹ The 1932 plants were ratooned in the spring of 1933.

HEREDITARY CHARACTER

The observations made at Bard, Calif., and at State College, N. Mex., indicate that in the strain of *Gossypium herbaceum* from Tashkent abnormal boll growth is a hereditary character, but apparently it is of such a nature that its expressions are largely influenced by environmental conditions. The effect of environment is shown by the increase in the percentage of bolls that produced supernumerary carpels and the development of intracapsulary bolls as the season advanced.

SUMMARY

The presence of supernumerary carpels in cotton bolls is not an uncommon abnormality in most types and strains of cultivated cottons. Usually the growth is limited to a white slender body extending through the center of the boll from the base almost to the apex. However, at Bard, Calif., in 1932 and 1933, this abnormal growth was observed in various degrees of development in a large proportion of the bolls of a strain of *Gossypium herbaceum* introduced from Tashkent, Uzbek, Union of Soviet Socialist Republics. In some of the bolls the growth was developed to the extent of forming small intracapsulary bolls containing from 1 to 3 mature seeds which were covered with lint of fair quality.

These intracapsulary bolls closely resembled the bolls in which they were contained in appearance, color, and shape. As viewed from outside they appeared to be divided into 2 to 4 carpels, but the placentae were not sufficiently developed to form separate carpels.

The abnormal boll growth observed in this strain of *Gossypium herbaceum* appears to be a hereditary character, but of such a nature that its expression is largely influenced by environmental conditions. The environmental effects are shown by the increase in the percentage of bolls containing supernumerary carpels and in the development of intracapsulary bolls as the season advanced.

A METHOD FOR ESTIMATING THE VOLATILE SULPHUR CONTENT AND PUNGENCY OF ONIONS¹

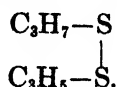
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INTRODUCTION

In studying the effect of various ecological conditions on the pungency of onions it became necessary to develop a method by means of which pungency could be measured more accurately than can be done by the ordinary tasting test.

It is generally known that onions and other members of the genus *Allium* owe their pungency and particular taste to an essential oil that is specific for each species. The volatile oil of *Allium cepa* L. was first investigated by Semmler² in 1892. He demonstrated the extremely high degree of pungency by showing that this oil occurs in onions in a concentration of only about 0.005 percent. After distilling 5,000 kg of onions he was able to obtain only 233 g of the crude oil. Determining some of the physical constants and the chemical composition of the crude oil, Semmler showed that the principal constituent of the oil is allyl-propyl-disulphide



The crude oil was found to contain 43.37 percent of sulphur, while the sulphur content of pure allyl-propyl-disulphide is 43.26 percent. Although Semmler studied some of the chemical properties of onion oil he did not develop any analytical procedure by which the oil could be measured quantitatively in different lots of onions.

DEVELOPMENT OF THE METHOD

A relatively simple method for determining the volatile sulphur in plants was proposed by Peterson.³ According to this method a stream of air is passed through a wide U tube containing the plant tissue and heated to 100° C.* The volatile sulphur is carried away by the air stream and is absorbed in a tube containing copper oxide filings which are thus reduced to copper sulphate. After the copper sulphate crystals have been dissolved the sulphate is determined by precipitation with barium chloride.

In order to test the dependability of this method two lots of 500 g of finely cut onion tissue were dried in a ventilated oven at 60° and at 100° C. for 24 hours. The dried residue was then hydrolyzed and the amount of volatile sulphur which remained was determined by

¹ Received for publication July 25, 1935; issued January 1936. Contribution 130 from Department of Vegetable Crops, New York (Cornell) Agricultural Experiment Station.

² SEMMLER, F. W. DAS ÄTHERISCHE OEL DER KÜCHENZWIEBEL (*ALLIUM CEPA* L.). Arch. der Pharm. 230: 443-448. 1892.

³ PETERSON, W. H. FORMS OF SULFUR IN PLANT MATERIALS AND THEIR VARIATION WITH THE SOIL SUPPLY. Jour. Amer. Chem. Soc. 36: 1290-1300. 1914.

the distillation method described later. It was found that heating at 60° had removed only 77.7 percent of the volatile sulphur originally present, and that heating at 100° had removed 88.3 percent. While this method may give dependable results with other plant tissues which are relatively low in volatile sulphur, it is obvious that it cannot be used to measure the pungency of onions.

Realizing the close chemical relationship between onion oil and mustard oil, the writer next investigated a method proposed by Dircks⁴ and improved by Schlicht⁵ which has become a standard method for the determination of mustard oil in rapeseed cakes. In this procedure the oil is first liberated from the glucoside by acid hydrolysis, then distilled into a solution of alkaline permanganate which readily oxidizes the sulphides of the oil to sulphate. The excess of permanganate is destroyed by the addition of alcohol, the precipitate of manganese dioxide is filtered off, and the sulphate is precipitated from the clear, acidified filtrate with barium chloride.

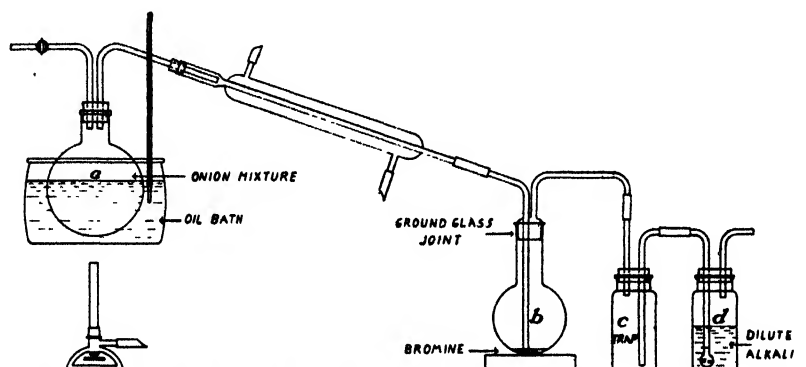


FIGURE 1.—Apparatus used in the determination of volatile sulphur in onions: a, 2-liter round-bottom, short-neck flask; b, 1-liter round-bottom flask containing 5 cc of bromine and 5 cc of water; c, trap; d, flask containing dilute sodium hydroxide for absorbing bromine fumes driven off during heating.

In applying this method to the determination of volatile sulphur in onions it was found that, while the results were satisfactory if proper precautions were taken, it was difficult to remove all traces of the manganese dioxide precipitate from the filtrate and also to prevent partial reduction of the sulphate after the addition of alcohol.

In an attempt to eliminate some of the difficulties encountered in using this method several other oxidizing agents were tried, and finally it was found that when the onion oil is distilled directly into saturated bromine water the sulphides present are rapidly oxidized to sulphate. The excess of bromine is easily driven off by heating, the small sulphur-free residue is filtered off, and the sulphate is precipitated from the filtrate with barium chloride. As compared with the permanganate method this procedure not only gave more accurate results but also required considerably less time. The details of this method are as follows:

Select a representative sample of at least 20 onions. Take one-fourth or one-half of each bulb and slice these sections into slices not

⁴ DIRCKS, V. ÜBER DAS VORKOMMEN DER MYRONSÄURE UND DIE BESTIMMUNG DES DARAUS GEBILDETEN SENFÖLS IN DEN SAMEN DER CRUCIFEREN UND IN DEN ÖLKUCHEN. Landw. Vers. Sta. 28: [179]-200, 1883.

⁵ SCHLICHT, A. EIN BEITRAG ZUR BEURTEILUNG DER RAPSKUCHEN NACH IHREM SENFÖLGEHALT. Landw. Vers. Sta. 41: [175]-190, illus.

thicker than one-half centimeter. Mix the material and weigh out exactly 500 g. Transfer the sliced material to a 2-liter, round-bottom, short-neck flask (fig. 1, *a*). Add 100 cc of concentrated hydrochloric acid and 250 cc of water. Submerge the flask in an oil bath to the level of the onion mixture and connect with a straight-tube condenser. To the lower end of the condenser attach a delivery tube which reaches to the bottom of a 1-liter, round-bottom flask (*b*) containing 5 cc of bromine and 5 cc of water. An outlet tube from the receiving flask leads to a trap (*c*) and from there to a flask (*d*) containing dilute sodium hydroxide to absorb bromine fumes which may be driven off during heating.

Heat the oil bath until it has reached a temperature of 122° to 124° C. Maintain at this temperature for 3 hours. During this period distillation should proceed very slowly and about 200 cc of the distillate should collect in the receiving flask *b*. At the end of 3 hours remove flask *a* containing the onion mixture from the oil bath; cool, and add 500 cc of water and 300 g of sodium chloride. While rotating the flask vigorously, add slowly, not more than 10 cc at a time, enough 50-percent sodium hydroxide almost to neutralize the mixture (about 95 cc) using litmus paper as an indicator. Again connect the flask to the condenser, heat the oil bath to 135° , maintain at this temperature for 1 hour, then raise the temperature to 145° and distill until 800 cc have collected in the receiving flask. Rinse the condenser and the delivery tube, stopper the receiving flask, and allow it to stand overnight. Add a few glass beads and heat very slowly on a Kjeldahl digestion rack or under a hood until all the bromine has been driven off. Transfer the distillate to a 1-liter beaker and evaporate on a steam bath to a volume of about 300 cc, or until the solution has become clear. Filter, wash the filter paper, and add 10 cc of bromine water to the filtrate. Heat on a hot plate or steam bath to drive off the excess of bromine and evaporate to a volume of about 300 cc. While still hot add, drop by drop, stirring vigorously, 10 cc of a 10-percent solution of barium chloride. Continue heating for 10 minutes. Cool, allow to stand overnight, filter through ashless filter paper, and wash the precipitate until free from chlorides. Ignite the precipitate and filter and weigh as barium sulphate. Make a blank determination if the reagents contain an appreciable amount of sulphur.

Precautions: All rubber tubing and stoppers used in the distilling apparatus should first be boiled in dilute alkali and then washed thoroughly to remove any adherent sulphur bloom. It is preferable to make connections by means of interchangeable ground-glass joints. This is almost essential for the connection between the delivery tube and the receiving flask (*b*). Where connections are made with rubber tubing the glass tubes should fit together as closely as possible to expose only a minimum of rubber to the bromine. Rubber stoppers can be protected effectively by a coat of acid-resistant stopcock grease.

A second outlet from the distilling flask (*a*) provided with a stopcock is useful in preventing possible sucking back of the distillate when the boiling flask is cooled.

EXPERIMENTAL RESULTS

In the following experiments, which were carried out in order to study the effect of modifying certain details of the procedure it was necessary to exclude the factor of individual variability of the sample. This was done conveniently by slicing 12 or more bulbs, thoroughly mixing the material, and weighing out 2 or 3 aliquots of 500 g each which were analyzed by slightly different methods.

EFFECT OF HYDROLYSIS ON THE YIELD OF VOLATILE SULPHUR

At the outset of the experiment it was noticed that the distillation of onion oil proceeds extremely slowly unless the heated material is first hydrolyzed with dilute hydrochloric acid. This suggests strongly that onion oil, like mustard oil, occurs in the bulb in the form of a glucoside which has to undergo hydrolysis before the oil can be distilled off. Table 1 shows that if hydrolysis is carried out by heating 500 g of sliced onions with 100 cc of concentrated hydrochloric acid and 250 cc of water, hydrolysis is complete within 3 hours. Where the hydrolysis was omitted prior to distillation the amount of sulphur in the first 800 cc of the distillate was reduced to less than one-fifth. On the other hand, extending the period of hydrolysis to 6 hours failed to give a higher yield of sulphur than was obtained after 3 hours hydrolysis.

TABLE 1.—*Effect of hydrolysis on the yield of volatile sulphur*

Sample	Sulphur in first 800 cc of distillate from 500 g of onion after -		
	No hydrolysis	3-hour hydrolysis	6-hour hydrolysis
A.....	Milligram 7.9	Milligram 56.1	Milligram 56.3
B.....	9.3	56.5	55.8

EFFECT OF HYDROGEN-ION CONCENTRATION ON THE YIELD OF SULPHUR IN THE DISTILLATE

Before the hydrolyzed oil can be distilled it is necessary partly to neutralize the mixture in order to prevent the distillation of appreciable quantities of hydrochloric acid which may later interfere with the proper precipitation of the sulphate. However, since protein sulphur is readily hydrolyzed in alkaline solution it is important to keep the mixture faintly acid. Table 2 shows that distillation from a neutral and an acid solution gave practically identical results, whereas the yield of sulphur was increased appreciably when the distillation was carried out from an alkaline solution.

TABLE 2.—*Effect of hydrogen-ion concentration on the yield of sulphur in the first 800 cc of the distillate from 500 g of onions*

Sample	Sulphur when approximate pH of mixture at the beginning of distillation was—		
	1.3	6.5	11.1
	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>
A.....	38.1	38.2	47.3
B.....	58.4	59.2	63.5

RATE OF DISTILLATION OF VOLATILE SULPHUR

The volatile sulphur is separated from the onion mixture by means of steam distillation. This process is usually slow, and in order to hasten the rate of distillation the boiling temperature of the onion mixture was raised by the addition of 300 g of sodium chloride. The temperature of the oil bath was maintained at 145° C.; a higher temperature did not seem advisable since Semmler⁶ found that onion oil decomposes at a temperature of 160°. In order to determine how much of the distillate should be collected to recover all of the volatile oil present, each 250 cc was collected separately during a few experiments.

Table 3 shows that the distillation of the sulphur is still incomplete after 1,500 cc of the liquid has been distilled. However, the quantity of sulphur in the distillate becomes very small after 750 or 1,000 cc has been distilled. Nevertheless, when it is desired to determine the actual amount of volatile sulphur it would be necessary to collect 2,000 cc or more of the distillate. On the other hand, when only comparative values are sought it seems hardly worth while to distill such a large amount, especially since the rate of distillation appears to be fairly uniform for different samples of onions. The writer in his experiments arbitrarily chose 800 cc as the quantity of distillate which was to be collected for the determination of relative pungency in onions.

TABLE 3.—*Rate of distillation of the volatile sulphur*

Successive fractions of 250 cc	Sulphur in successive fractions of the distillate		Successive fractions of 250 cc	Sulphur in successive fractions of the distillate	
	Sample E	Sample F		Sample E	Sample F
	<i>Milligrams</i>	<i>Milligrams</i>		<i>Milligrams</i>	<i>Milligrams</i>
First.....	64.34	50.06	Fourth.....	1.21	0.65
Second.....	7.52	4.56	Fifth.....	.70	.49
Third.....	2.57	1.44	Sixth.....	.54	.30

PRECISION OF THE METHOD AND VARIABILITY OF THE SAMPLE

To obtain some idea concerning the precision of the method, as well as the variability of the sample which may be expected, a commercial lot of Yellow Bermuda onions was divided into 5 groups, each containing 10 bulbs. Each group was prepared for analysis separately,

⁶ SEMMLER, F. W. See footnote 2.

and after slicing and mixing had been completed, these lots were divided again into 2 separate samples of 500 g each, designated as A and B (table 4). Differences between the analyses of A and B illustrate the precision of the method while the successive numbers of the samples signify the variability of the samples.

TABLE 4.—Volatile sulphur determinations on 5 samples of the same lot of Yellow Bermuda onions illustrating precision of the method and variability of the sample

Item	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Average
	All-quot A	All-quot B	All-quot A	All-quot B	All-quot A	All-quot B	All-quot A	All-quot B	All-quot A	All-quot B	
Sulphur.....	P.p.m. 110	P.p.m. 108	P.p.m. 116	P.p.m. 117	P.p.m. 109	P.p.m. 111	P.p.m. 115	P.p.m. 116	P.p.m. 119	P.p.m. 122	114.3

Table 4 shows that in every case the error due to the variability of the sample was greater than the experimental error. Unless a greater uniformity of the sample can be assured it seems advisable to take a larger number of bulbs, perhaps 20 or 25, in order to obtain more accurate results. At any rate, it appears doubtful whether differences of less than 10 parts per million are significant. It must be realized, however, that differences in the content of volatile sulphur between samples from several varieties were as a rule very much greater. While some samples contained as little as 28 parts per million others yielded as high as 200 parts per million.

DISCUSSION

The proposed method for estimating the relative pungency of onions is based on the assumption that the onion oil has a definite composition and that differences in the pungency of onions are due solely to quantitative differences in the percentage of oil present. This assumption seems justified since Semmler found that the onion oil consists almost entirely of allyl-propyl-disulphide, a definite chemical compound. Furthermore, the writer and Knott⁷ found that wherever differences in pungency could be detected by the tasting test they were associated with corresponding differences in the content of volatile sulphur.

As previously shown, during the distillation of volatile sulphur, more than 1,500 cc of the liquid must be distilled before all traces of volatile sulphur are removed from the onion mixture. It will be noticed in table 3, however, that after the first 1,000 cc have been distilled the quantity of sulphur in the subsequent portions of the distillate rapidly approaches a constant value, which in comparison with the total amount present is exceedingly small. It seems possible that these last traces of volatile sulphur are not derived from onion oil but are due to a second reaction, possibly a very slow decomposition of protein sulphur. At least, the trend of the distillation curve suggests this explanation.

The principal advantage of the proposed method is that in studying the factors determining the pungency of onions results can be expressed

⁷ PLATENIUS, H., and KNOTT, J. E. PUNGENCY OF ONIONS IN RELATION TO VARIETY AND ECOLOGICAL FACTORS. Amer. Soc. Hort. Sci. Proc. (1934) 32: 593-595. 1935.

in numerical values which make it possible to compare onions of different varieties or bulbs grown under different ecological conditions. Preliminary experiments indicate that the same method, with slight modifications can be used to determine the pungency and the volatile sulphur content of garlic, cabbage, and possibly other vegetables which owe their specific taste to some volatile oil containing sulphur.

While the method itself is relatively simple it is time-consuming because of the slow process of steam distillation. Therefore it cannot be recommended as a routine method where large numbers of samples are involved.

SUMMARY

A new and relatively simple method is described for estimating the volatile sulphur content and pungency of onions. This method is based on the assumption that onion oil has a definite chemical composition, and that differences in the pungency of onions are due solely to quantitative differences in the amount of oil present and, indirectly, to the volatile sulphur content. The method possesses sufficient accuracy for the purpose, but since it involves steam distillation it is time-consuming and cannot be recommended as routine procedure when a large number of samples is involved.

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HISTOLOGY OF THE CARYOPSIS OF YELLOW DENT¹ CORN, WITH REFERENCE TO RESISTANCE AND SUSCEPTIBILITY TO KERNEL ROTS¹

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INTRODUCTION

Inasmuch as the kernel of corn (*Zea mays* L.) is susceptible to a number of ear rot organisms, it has seemed advisable to obtain a picture of the histology of the normal spikelet and kernel as a basis for the study of various phases of the disease problem. This paper, therefore, outlines briefly the development and the microscopic anatomy of the caryopsis of dent corn and is concerned primarily with those structures that may have a bearing on the problem of host resistance or susceptibility to fungus diseases in general and to invasion by *Diplodia zeae* (Schw.) Lév. in particular.

MATERIAL AND METHODS

The hand-pollinated yellow dent corn used in this study was obtained from the plots of the departments of plant pathology and genetics at the University of Wisconsin and from J. R. Holbert, of Bloomington, Ill. Kernels both from strains that had shown themselves over a period of years to be resistant to ear rots in the field and from those that under comparable conditions had proved susceptible were collected during the summers of 1930 to 1933, inclusive. The first collections were made 2 days after pollination. Specimens were taken at intervals of from 2 to 7 days during the earlier periods of development of the kernel, killed in medium chromo-acetic solution, formol acetic alcohol, or Gilson's or Juel's zinc chloride fixative, and embedded in paraffin by the alcohol-chloroform method.³ Before fixation, the healthy mature kernels were soaked in water overnight, and when softened the sides or distal ends of the kernels were trimmed so as to expose portions of the starchy endosperm to facilitate the penetration of the various fluids.

A simple method of overcoming the difficulty usually experienced in sectioning kernels approaching maturity is to soak the embedded material in water for from a few hours to overnight, after having trimmed the paraffin block in such a manner as to expose the tissue

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² The writer gratefully acknowledges her indebtedness to J. R. Holbert, M. R. Harris, and A. L. Smith for supplying material from their corn plots at Bloomington, Ill., and Madison, Wis.; to Boyd C. Frye for his painstaking collecting of specimens at stated intervals at Bloomington, Ill.; and to Eugene H. Herrling, of the Department of Plant Pathology, University of Wisconsin, for all the photography involved in the problem. Thanks are also due J. G. Dickson for helpful suggestions during preparation of the manuscript.

³ The material embedded during the four seasons included specimens from inbred strains A, A48, A9, A956, Brio, R4, R313, L, and Lan; single crosses nos. 365, 48, and 58; and top crosses Krug X A48 and Krug X Lan.

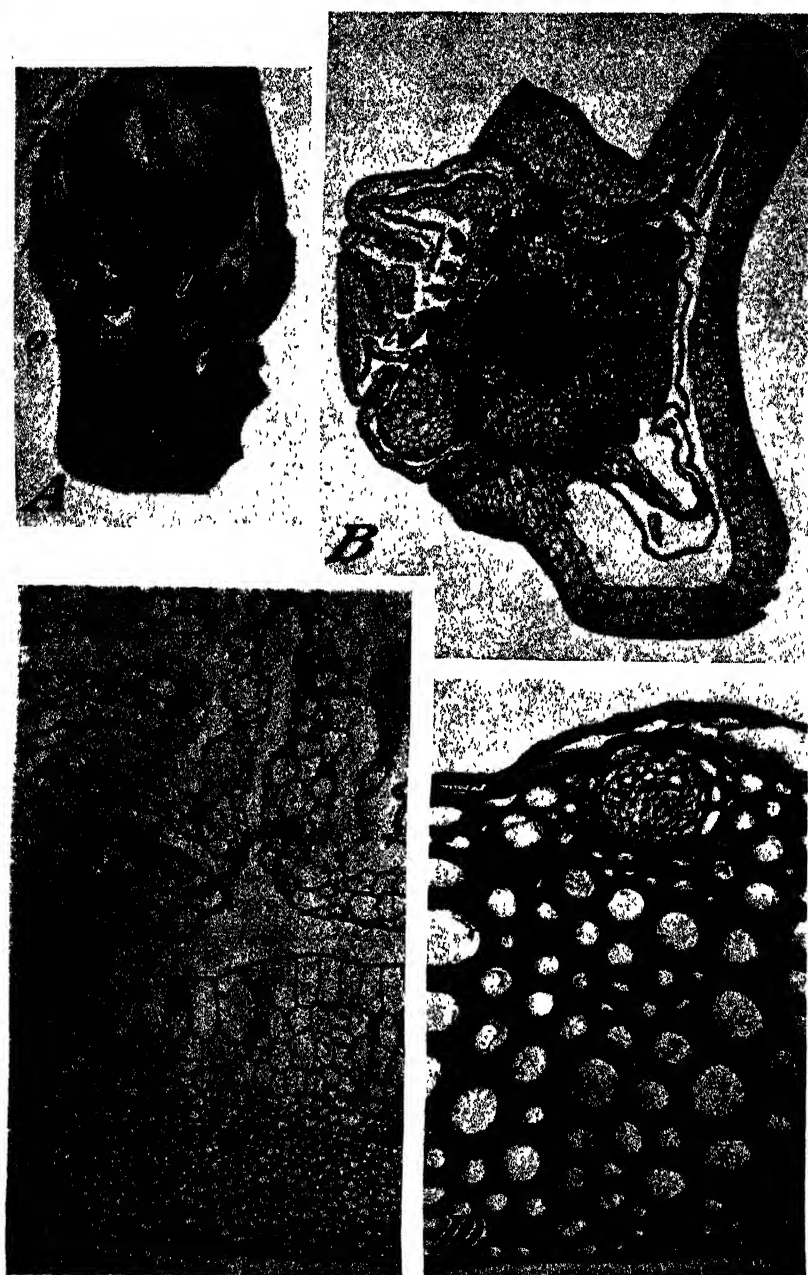


FIGURE 1.—The pistillate spikelet.

FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

on the side to be cut. Seldom, however, can an entire kernel be successfully sectioned at one time, hence intermittent soaking and cutting are usually necessary. The sections were cut to a thickness of 8μ to 10μ . This method provided better sections than were obtained by the use of a hydrofluoric acid treatment as recommended for wheat kernels in an earlier paper (7).⁴

The principal stains used were Flemming's triple combination or safranine and light green following either chromo-acetic or formol acetic alcohol fixative in the case of healthy kernels, and Delafield's haematoxylin, safranine, and orange G after Gilson's or Juel's fixative when dealing with infected kernels. Sudan III served to indicate the presence of suberized membranes. In case of a positive reaction to this stain, insolubility of the parts in question in 72-percent H_2SO_4 was considered additional evidence of the presence of suberinlike substances.

The illustrations presented are unretouched photographs and photomicrographs.

HISTOLOGY OF CORN KERNEL

PISTILLATE SPIKELET

It is well known that the pistillate spikelet of dent corn consists of two flowers, the basal of which is usually rudimentary and non-functional. The aborted flower contains a rudimentary pistil and three rudimentary stamens enclosed by two thin folded glumes, the palea and lemma. In the fertile flower there are rudimentary stamens and a functional ovary, also encircled by palea and lemma, the whole being surrounded by the heavier outer glumes (fig. 1, *A, B, C, D*). These glumes constitute the chaff of the cob and mature grain.

At flowering time, the ovary wall is composed of a number of rows of thin-walled cells containing considerable starch, an outer epidermal layer, and a somewhat thinner-walled inner epidermis. Two strands from the vascular elements of the pedicel traverse the wall on the germinal side of the ovary and pass into the two lobes of the style. In the style there is also a group of nonvascular cells that parallels each vascular bundle on its inner side. At the top of the ovary these groups of cells leave their positions along the vascular bundles, cross the ovary wall, and terminate on its inner surface near the top of the ovular cavity (fig. 2, *A, B*), providing a pathway along which the pollen tube travels to the cavity of the ovary. Miller (6, p. 258) speaks of them as "sheathlike" cells and Randolph (8) finds an explanation for their presence in the manner in which the three carpels have become fused to form the compound pistil. She considers the strands to be composed largely of epidermal cells. The

⁴ Reference is made by number (italic) to Literature Cited, p. 882.

EXPLANATORY LEGEND FOR FIGURE 1

FIGURE 1. A, B, C, D, Cross section through a spikelet at the base of the ovary. A, a, The outer glume subtending the aborted flower has been removed. Spikelet fixed 7 days after pollination; o, Outer glume; l, lemma of fertile flower; l', lemma of aborted flower; a and a', rudimentary anthers of fertile and aborted flowers. Stained with triple stain. X 10. C, Portion of a cross section of a spikelet, fixed 2 days after pollination, showing the structure of the outer glume (o) and the structure of and folding of the inner glumes (i). Stained with triple stain. X 54. D, Portion of a cross section of an outer glume, fixed 27 days after pollination. The walls of the cells beyond the vascular elements have become much thickened; those within the line of bundles have collapsed, leaving the vascular elements in a position near the inner margin of the glume. Stained with triple stain. X 136.



FIGURE 2.—Sections from the top of the ovary. *A*, A portion of a longitudinal section of an ovary, fixed 2 days after pollination. The ovary wall is cut in a plane that includes parts of the styler canal and of the group of "sheathlike" cells, along which the pollen tube travels to the cavity of the ovary, and one of the two vascular strands that extend into the silk. *st*, Styler canal; *s*, sheathlike cells; *v*, vascular elements; *c*, cone-shaped thickening of the outer integument at the base of the styler canal; *i*, inner integument; *n*, nucellus. Stained with safranin and light green. $\times 120$. *B*, Portion of a cross section of the ovary, showing the relative positions of the vascular elements and the sheathlike cells in the ovary wall near the base of the style: *v*, Vascular elements; *s*, sheathlike cells. Stained with triple stain. $\times 70$. *C*, Detail of the cone-shaped folding of the outer integument at the base of the styler canal. This section is from the ovary shown in *A*; *c*, *i*, and *n* as in *A*. Stained with safranin and light green. $\times 450$.

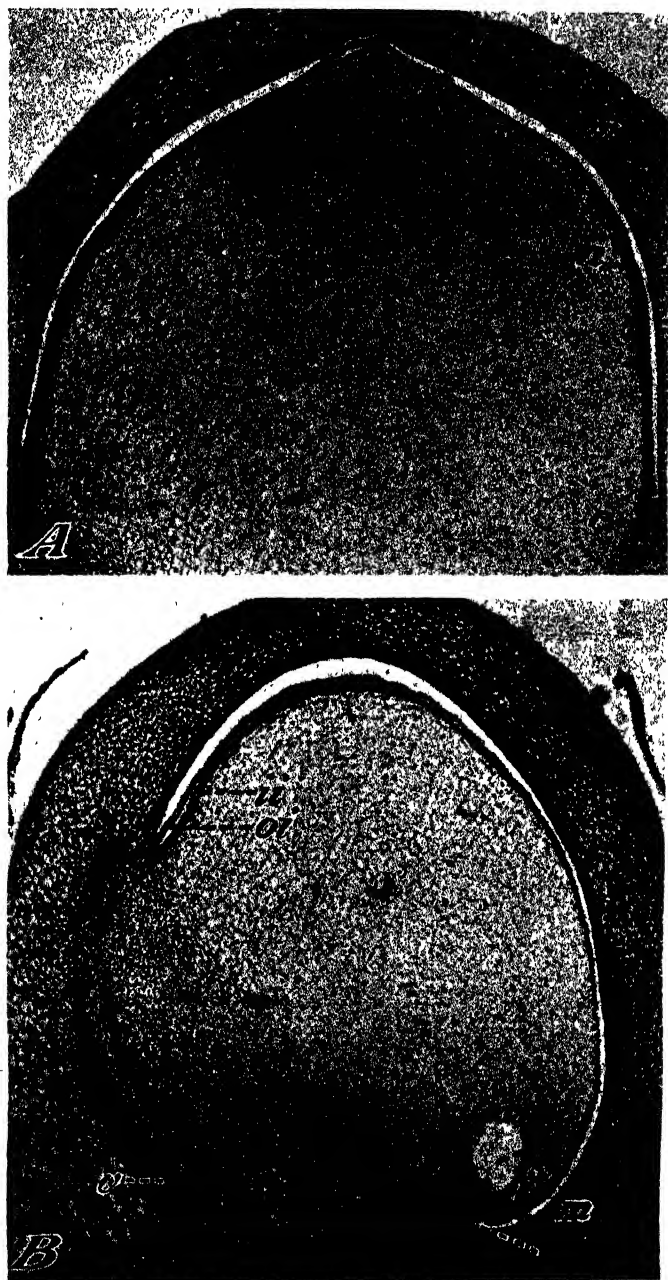


FIGURE 3.—Longitudinal sections of ovaries, fixed 2 days after pollination. *A*, Section cut parallel to the germinal side of the ovary; *oi*, outer integument, showing the cone-shaped protuberance that projects into the depression of the stylar canal; *ii*, inner integument; *w*, wall of ovary; *n*, nucellus; *c*, chalasa. Stained with triple stain. $\times 50$. *B*, Near-longitudinal section cut approximately at right angles to *A*; *ii*, inner integument; *oi*, outer integument; *e*, embryo sac; *m*, micropyle; *v*, vascular elements. Stained with safranine and light green. $\times 42$.

modified campylotropous ovule is attached for approximately one-third of its surface to the bottom of the cavity of the ovary (fig. 3).

The integuments of the ovule are layers of thin-walled tissue for the most part two cells thick, except at the micropyle, where the

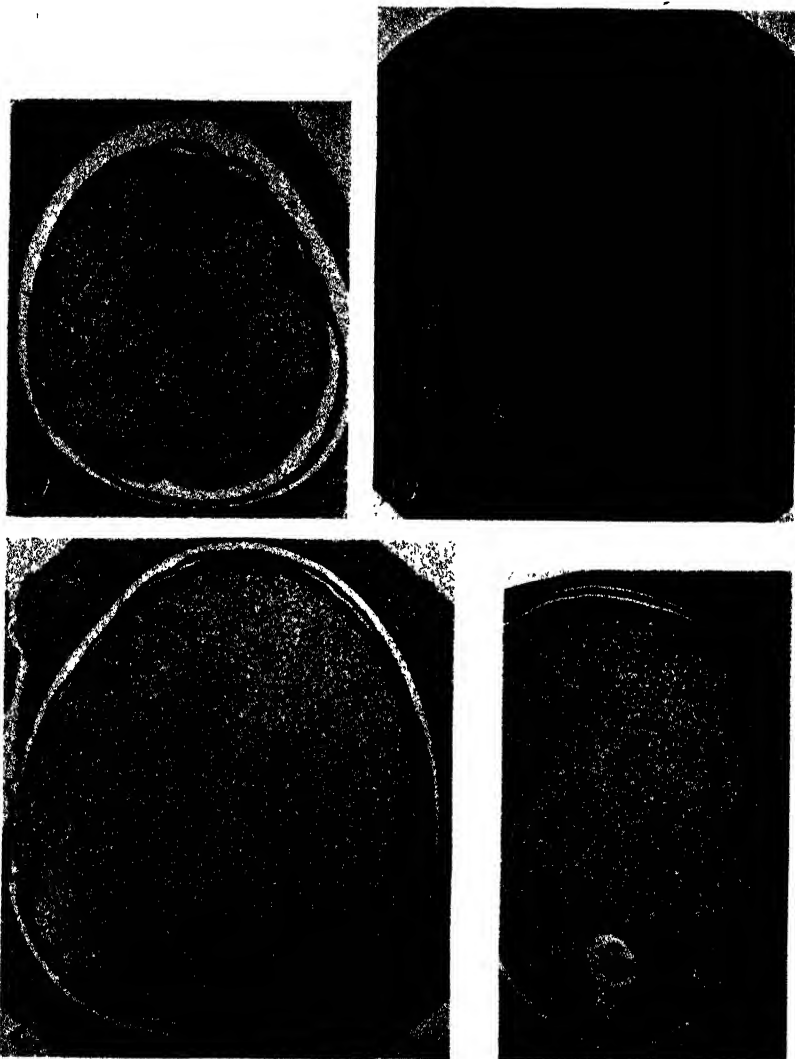


FIGURE 4.—Cross sections from a single ovary, showing the extent of the outer integument and the exposed surface of the inner integument at four different levels. *A*, Near the top of the ovary; *B*, at the equator; *C*, through the antipodal cells of the embryo sac; *D*, in the region of the fertilized egg cell. *e*, Ends of outer integument; *f*, surface of inner integument not covered by outer integument. Stained with triple stain. $\times 35$.

inner integument especially becomes somewhat club-shaped in section. Neither the integuments nor the nucellus contain any starch, though at the stage of development shown in figures 3 and 4 the cells of the ovary wall and the pedicel of the floret possess an abundance of that

food material (figs. 6, *B*, and 15, *A*). The inner integument covers the free surface of the ovule completely with the exception of the micropylar orifice and usually is in contact with the epidermis of the

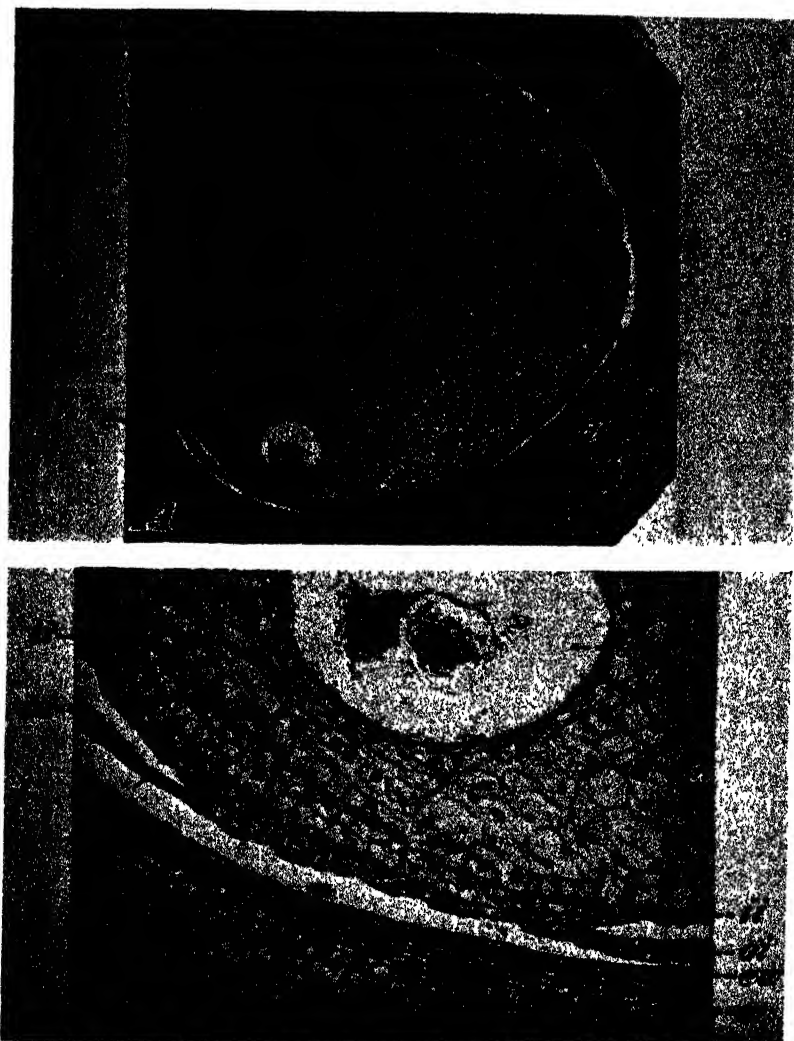


FIGURE 5.—Cross section of an ovary, showing the tissues in the region of the embryo sac. *A*, Ovary fixed 2 days after pollination, showing, in the absence of a funiculus, the similarity at that stage of development between the nucellar cells and the placental tissue of the ovary wall; *c*, chalaza; *pl*, placental tissue; *w*, wall of ovary; *n*, nucellus; *ii*, inner integument; *oi*, outer integument. Stained with triple stain. $\times 42$. *B*, Detail of *A* in the region of the embryo sac, showing the extent of the outer integument in this ovary; *ii*, inner integument; *oi*, outer integument; *e*, embryo sac; *n*, nucellus; *ew*, inner epidermis of the ovary wall (*w*). $\times 208$.

nucellus. The outer integument, which originates alongside of the inner integument, occupies a parallel position throughout its extent except for a cone-shaped folding which projects into the depression of the so-called "stylar canal" at the distal end of the ovary (figs. 2, *C*; 3, *A*, *B*).

Concerning the extent of the outer integument, several writers have shown considerable unanimity of opinion. True (10, p. 215) states that "The outer integument is incomplete, failing to cover an area extending from the micropyle to the base of the style, in length, and in width equal to about one-half the diameter of the ovule." Weatherwax (11, p. 491) says: "The one part of the outer integument seldom grows further than the top of the ovary, where it forms a folded or wedge-shaped body closing the stylar canal." Miller (6, p. 258) states that "the outer coat of the ovule is incomplete and extends about half way around it", and Haddad (3, p. 7) is of the opinion that "the outer integument extends only a short distance beyond the stylar canal where it terminates."

Serial longitudinal and cross sections of entire ovaries, prepared in the course of this study, indicate that the outer integument covers more of the surface of the inner integument than the statements just quoted might suggest (figs. 4, 5, 6, B). Although there were some variations in the extent of the outer integument in the different ovaries, in all cases examined the exposed surface of the inner integument appeared to be a comparatively narrow, more or less triangular area. The apex of the triangle was to be found near the point where the sheathlike cells of the style terminate in the ovary wall and its base below the micropyle. In sections fixed as early as 2 or 3 days after fertilization, it is often difficult to determine the limits of the outer integument because of degenerative changes in both integuments which result in the two structures appearing to be more or less unified.

The nucellus, in which the embryo sac is embedded, consists of a delicate parenchymatous tissue bordered on its free surface by a distinct but thin-walled epidermal layer. Before fertilization the nucellus and what may be called the funicular-hilar-placental region—although there is neither funiculus nor true hilum—merge into each other without any distinctly marked cell differences (fig. 5, A).

DEVELOPMENT OF KERNEL

EMBRYO

Development of the kernel begins almost immediately after fertilization, and growth proceeds rapidly. According to Miller (6), fertilization takes place 26 to 28 hours after pollination, and traces of the pollen tube may be visible within the embryo sac for some time thereafter. He notes the rapid growth of the endosperm and states that the cells of the endosperm may fill the embryo sac when the embryo numbers only 14 to 16 cells.

In sections of spikelets fixed by the writer 2 days after pollen had been applied to the silk of the ear, the pollen tube, staining a deep red with safranine, was visible in the micropyle and embryo sac. In most cases fertilization had taken place. Sections of slightly older spikelets indicated that division of the endosperm nucleus had begun almost immediately after fertilization; they also showed numerous free nuclei formed before cell walls appeared and a more rapid division of the endosperm than of the egg nucleus (fig. 6, A). When the developing endosperm had almost filled the enlarged nucellar space the embryo still remained comparatively small (fig. 7, C), although in a nearly mature caryopsis it occupies approximately one-third to one-half of the surface of a median longitudinal section of a kernel (fig. 8).

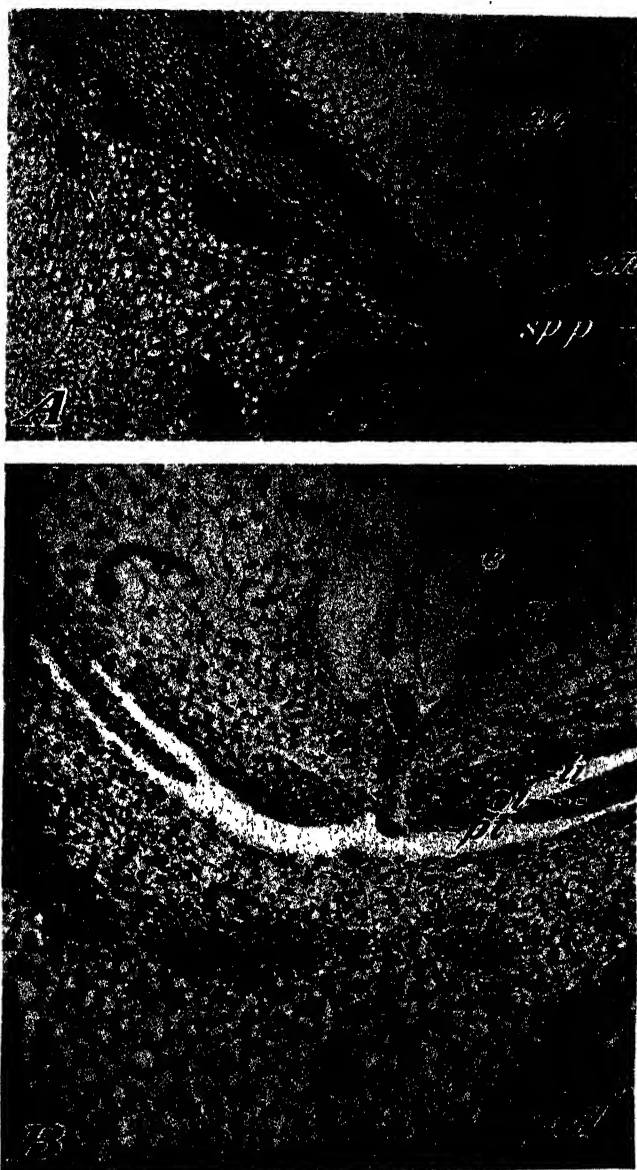


FIGURE 6.—Portions of longitudinal sections of developing kernels, showing tissues in the regions of the embryo. *A*, Portion of a longitudinal section of a developing kernel, showing the more rapid growth of the endosperm than of the embryo. Strain A, grown at Madison, Wis.; open-pollinated; fixed on August 15, at which time the silks were still green: *n*, Nucellus; *en*, endosperm; *em*, embryo; *sp*, spongy pericarp. Stained with triple stain. $\times 48$. *B*, Portion of a longitudinal section of an ovary, showing the relative positions of the two integuments at the micropyle. Strain A48, grown at Madison, Wis.; fixed on August 15, 3 days after pollination: *e*, Embryo sac; *n*, nucellus; *ti*, inner integument; *oi*, outer integument; *pt*, pollen tube; *s*, starch granules; *ped*, pedicel. Stained with triple stain. $\times 125$.

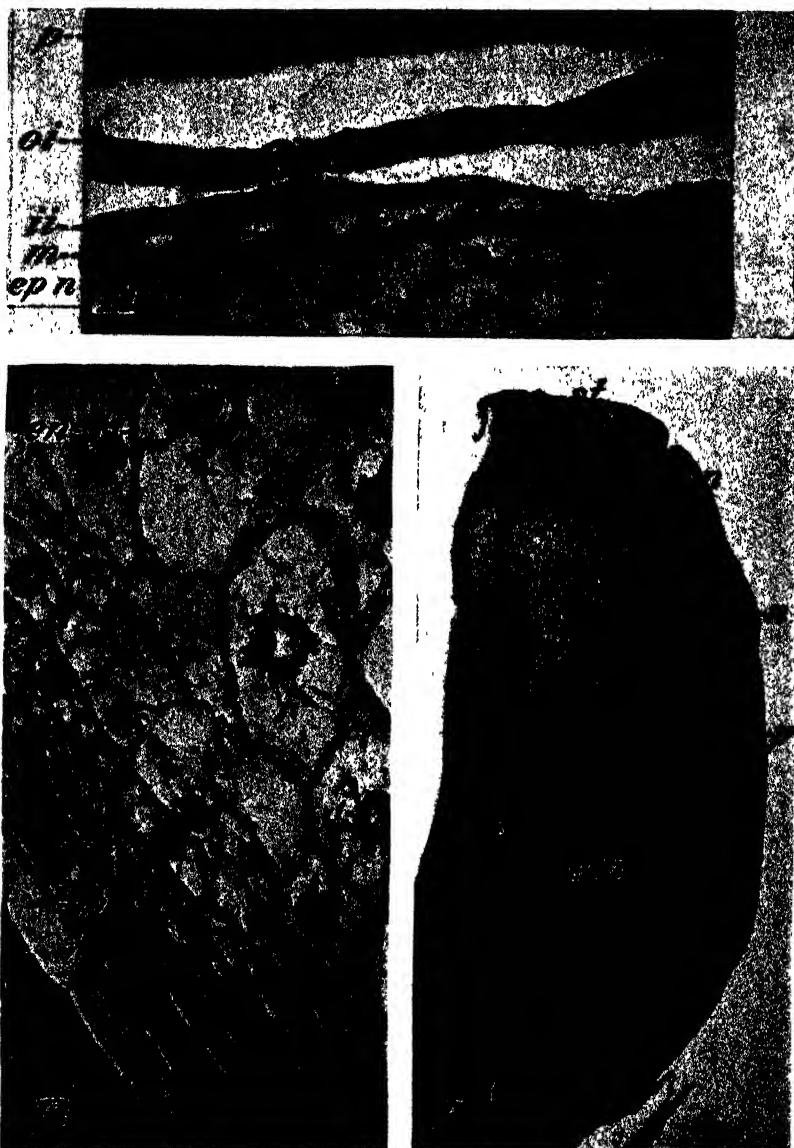


FIGURE 7.—Developmental phases of the corn kernel. *A*, Portion of a cross section of a developing kernel of strain A48, fixed 5 days after pollination, showing degenerative changes in both integuments: *ep*, *n*, Epidermis of nucellus; *m*, line along which the suberized membranes of the testa will appear; *ii*, inner integument; *of*, outer integument; *p*, pericarp. Stained with triple stain. $\times 393$. *B*, Detail of *C*. A portion of the endosperm between the embryo and style. At this stage of development, cell division seems to be more active in this region than in other parts of the endosperm; little starch is to be found in the endosperm. *en*, Endosperm; *al*, aleurone layer (immature); *n*, remains of nucellus; *i*, remnant of the integuments; *p*, pericarp. Stained with triple stain. $\times 203$. *C*, Longitudinal section, showing the relative sizes of the embryo and endosperm in a developing kernel of strain A, open-pollinated, fixed on August 23 at Madison, Wis.: *st*, Remnant of the style; *sc*, stylar canal; *en*, endosperm; *n*, remnant of the nucellus; *p*, pericarp; *em*, embryo; *c*, chalaza; *c-c*, hilar opening through the integument. Stained with triple stain. $\times 13$.

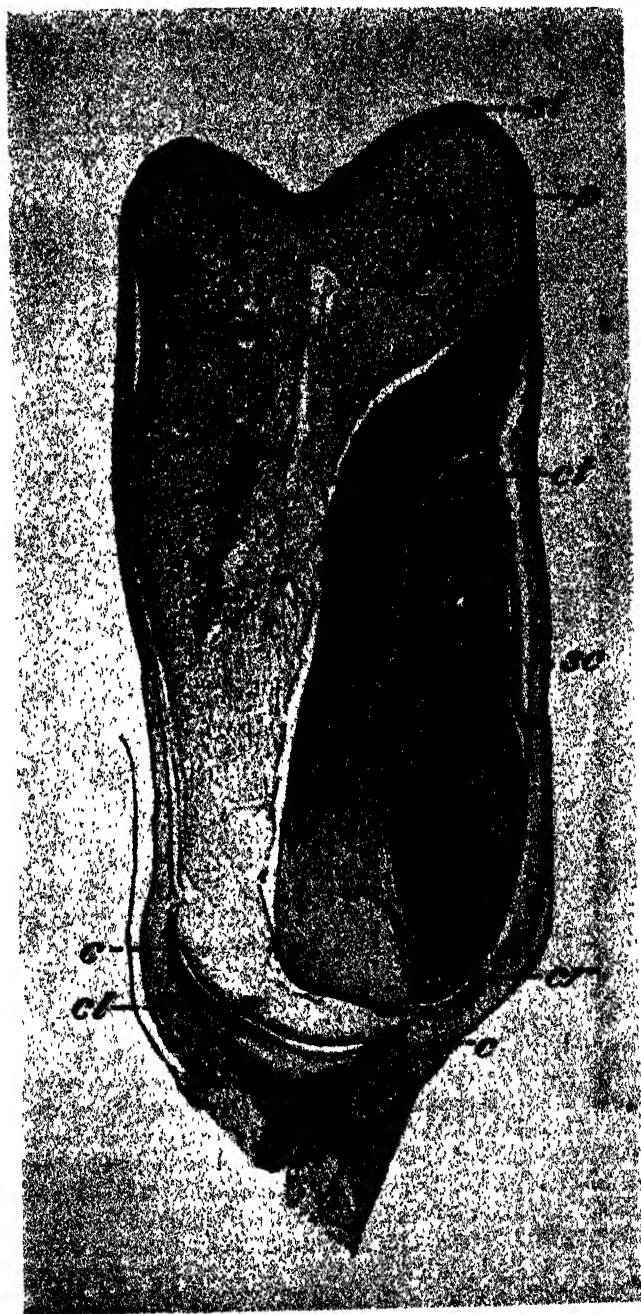


FIGURE 8.—Longitudinal section of a maturing kernel of an L inbred grown at Madison, Wis.; fixed on September 15, 42 days after pollination. In this kernel the embryo appears to be fully formed; the cells of the endosperm are not yet completely filled with starch and the closing layer extends only part way across the hilar orifice (c-c). *st*, Remnant of the style; *p*, pericarp; *en*, endosperm; *cl*, coleoptile; *sc*, scutellum; *c*, chalassa; *cr*, coleorhiza; *cl*, closing layer of the hilar orifice. Stained with triple stain. $\times 14$.

INTEGUMENTS

Degenerative changes in the integuments also begin about the time of fertilization. The literature on the subject has shown a variety of opinions concerning the modifications which take place in the epidermis of the nucellus and in the inner integument of the ovule during the maturation of the Caryopsis of corn. True (10, p. 217) speaks of the strongly cutinized outer walls of the nucellar epidermis. Concerning the integuments he says:

Previous to the collapse of the outer integument, the inner integument shows little change. Shortly, however, a slight tendency to weaken is seen, especially in the external layer of cells.

Robbins (9, p. 173) lists as a part of the mature grain, "Testa, inner integument of two layers." On the other hand, Randolph (8, p. 6) states:

The absorption of the tissues in question was followed in a close series of stages, and in all the mature kernels studied there was in most cases no nucellar or integument tissue, the aleurone layer of the endosperm lying in close contact with the inner epidermis of the pericarp. In some scattered places a little unabsorbed material was seen, but it never formed a definite layer.

Such statements, indicating that the outer integument in the corn ovary disappears before the inner, as is the case in wheat, or that in the mature kernel there is no layer derived from the integuments, have not been supported by sections examined in the course of this study. On the contrary, certain sections showed more rapid degeneration or resorption of the inner than the outer integument. From other sections it appeared that there had been little, if any, difference in point of time in the disintegration of the two integuments⁵ (fig. 7, A; fig. 15, A). These sections also showed the presence of a thin suberized layer, the semipermeable membrane of the testa, which appeared early in the development of the kernel along the inner surface of the inner integument in contact with the nucellus (fig. 7, A). It seems that the suberin was laid down in close contact with the nucellus and inner integument only after degenerative changes in the latter had begun. Attempts to demonstrate the presence of suberin on either of the adjacent walls when the epidermis of the nucellus and the inner integument were separable were unsuccessful. Thus, the origin of the membrane may be open to question. It seems probable, however, that the suberized layer in the corn kernel is derived from the integument, as is the case in wheat (*Triticum vulgare* Vill.)⁶ (?), bluegrass (*Poa pratensis* L.) (1), and Johnson grass (*Sorghum halepense* (L.) Pers.) (4), although the number and position of the layers vary with the different genera. In the wheat kernel there are two suberized layers in the testa. They are both laid down on the inner integument, the outer layer becoming much the thicker. In the mature kernel this layer is not of uniform thickness throughout its extent, being visibly thinner in the section over the embryo than in the groove and at the distal end of the

⁵ The chances are that Haddad (3, pp. 9, 11) also pictures such a condition, although the legend for his figure 4 suggests a different interpretation. From a comparison of his figures 2 and 4 it seems probable that what is considered to be the remains of the outer integument (fig. 4, 2) is the inner layer of the ovary wall and that both degenerating integuments, rather than the inner one only, are shown in his figure 4, 3.

⁶ According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L., but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

kernel (7). In the case of *Poa compressa* L. and *P. pratensis* L. the picture is somewhat different. Anderson (1, p. 1016) states:

Soon after fertilization a layer of suberin is found in the cell walls of the inner integument adjacent to the nucellus, and in the cell walls of the outer integument adjacent to the inner integument. Usually the inner suberized layer develops first.

In the mature caryopsis she finds:

The inner layer of the inner integument consists of a comparatively thin layer of suberin. Its remaining cell walls and their contents are compressed. The inner layer of the outer integument adjacent to the inner integument is composed of a thick layer of suberin. The remaining cells and their contents are presumably dissolved.

In Johnson grass, according to Harrington and Crocker (4, p. 220), it is the inner wall of the inner integument that is most highly suberized, although the various layers of the pericarp also contain suberin.

In the corn kernel the membrane is very thin, thinner in some cases than the suberized layer covering the tip of the adjacent coleoptile (fig. 9). It is so thin that variations in thickness that may exist are not easily seen. However, in section it appears to be even more slender for a short distance above the chalaza and over the embryo than elsewhere over the surface of the seed, and slight differences in color intensity when stained with Sudan III suggest the probability that less suberin is present in these areas than at the distal end of the kernel. Treatment of the kernels with iodine-potassium iodide solution also indicates that the membrane is most permeable at the tip of the kernel in the region of the embryo. Furthermore, the parts of the membrane over the embryo and encircling the kernel just above the chalaza seem to be those most easily pierced by fungus hyphae. Kernels carrying a moderately severe diplodia infection show dense masses of hyphae on both sides of the membrane in these regions, while the closed hilar orifice and the distal parts of the kernel may be comparatively free of fungus. Whether the membrane is a thin layer of suberin deposited along the wall of the inner integument, as in the case of the wheat kernel (7), or whether the entire marginal wall itself becomes impregnated with suberin was not determined. With the exception of this one suberized layer the integuments practically disappear before the kernel is mature.

NUCELLUS

During the rapid enlargement of the ovary following fertilization the nucellar cells also increase in size. They soon break down and disappear, however, as the rapidly growing endosperm pushes out to occupy the nucellar space (figs. 6, A, and 7, B, C), the epidermis being the last portion of the nucellus to lose its identity (fig. 10, A). There seems to be little question that remains of the nucellus are to be found in nearly all kernels, although it is not so clear whether a continuous layer coextensive with the testa, similar to that found in wheat, is the rule. The crushed nucellar cells form a hyaline thread or ribbon in such close contact with the outer walls of the aleurone cells that the two usually appear to be one. Nevertheless, staining reactions occasionally gave a hint of the dual nature of these walls (fig. 10, B), while variations in their apparent thickness suggested the same idea.

Infrequently a division of the wall may be observed also. In one kernel from a supposedly mature ear tested on the germinator the sections were torn in such a way that these broad walls split and a

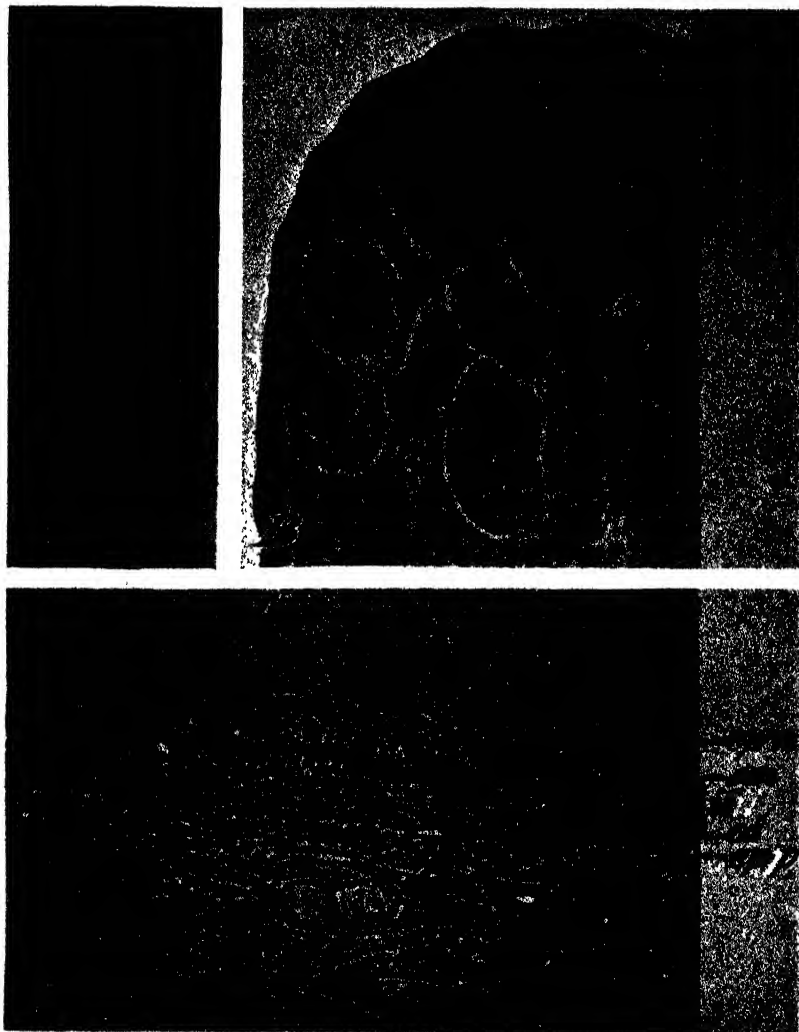


FIGURE 9.—The suberized membrane of the testa. *A* and *B*, Sections from a maturing kernel of inbred Lan grown at Bloomington, Ill., showing the relative thickness of the suberized membrane of the testa over the embryo (*A*) and the suberized layer over the tip of the coleoptile (*B*). Stained with Sudan III, mounted in glycerin. $\times 535$. *C*, Portion of a cross section of a developing kernel of strain A, open-pollinated; fixed on August 19, at which time the silk was beginning to brown. This section is located above the micropyle and shows little of the outer integument. *ep. n.*, Epidermis of nucellus; *sm*, suberized membrane; *il*, inner integument; *oi*, outer integument; *ep. p.*, inner epidermis of pericarp. Stained with Sudan III, mounted in glycerin. $\times 356$.

long narrow thread remained adhering to the suberized layer of the testa, although the encircling walls of the aleurone cells were not ruptured (fig. 10, *C*).

HILUM REGION

In their study of the caryopsis of Johnson grass and of Sudan grass, Harrington and Crocker (4) describe parts that bear a close resemblance to those found in the hilar region in corn. The protective

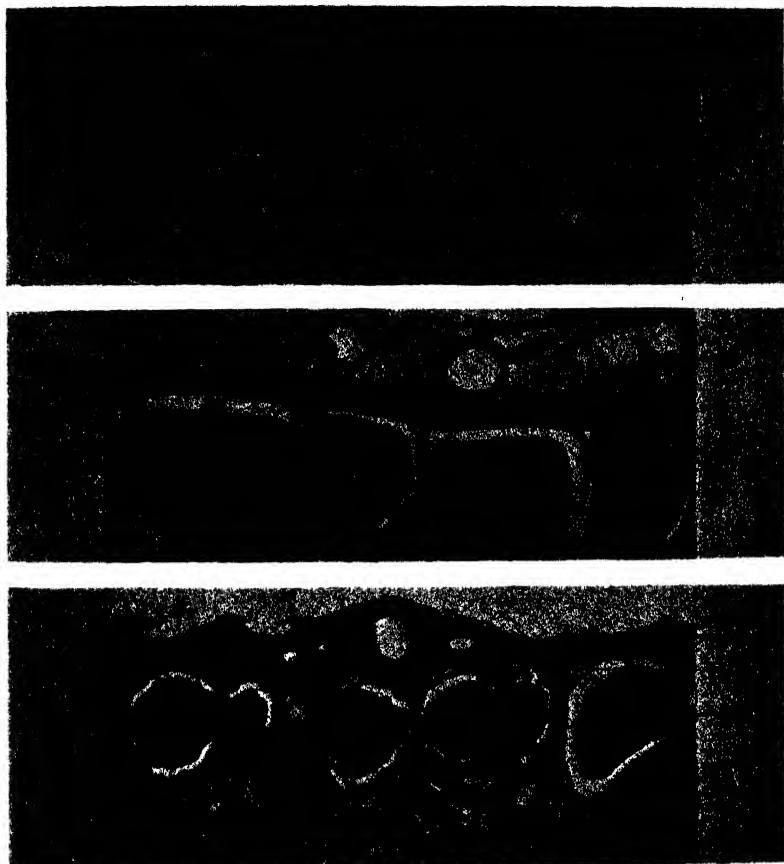


FIGURE 10.—Sections showing remnants of the nucellus. *A*, Portion of a cross section of a developing kernel of strain A, open-pollinated, grown at Madison, Wis., and fixed on September 6: *p*, Pericarp; *ep*, *p*, inner epidermis of pericarp; *sm*, suberized membrane of testa; *ep*, *n*, epidermis of nucellus; *al*, aleurone layer. Stained with triple stain. $\times 335$. *B*, Portion of a longitudinal section of the kernel of inbred Lan shown in figure 9, *A*, *B*. The double nature, in some places, of the broad outer walls of the aleurone cells suggests the presence of remnants of nucellar tissue. *p*, Pericarp; *sm*, suberized membrane of testa; *n*, remnant of nucellar tissue; *w*, *al*, wall of aleurone cell; *al*, aleurone layer. Stained with Sudan III and gentian violet, mounted in glycerin. $\times 445$. *C*, Portion of a longitudinal section of a dormant, supposedly mature kernel of a Golden Glow strain grown at Madison, Wis., showing what appears to be the remains of the nucellus adhering to the hyaline suberized membrane of the testa: *sm*, Suberized membrane of testa; *n*, nucellar tissue; *al*, aleurone layer; *en*, endosperm. Stained with Delafield's haematoxylin, safranin, and orange G. $\times 345$.

layers are relatively thicker in the grasses than in corn, but otherwise the similarity is striking. They state (4, p. 200):

Since in a caryopsis the seed never becomes detached from its pericarp, there is, of course, no true hilum, or seed scar. There is however in the caryopsis of the *Andropogonae*, a large opening through the inner integument in the position corresponding to the hilum.

They further state (4, p. 219):

The large circular hilar orifice contains no vascular elements, conduction from the vascular bundle of the pedicel and outer layers of the pericarp over the hilar region being by means of parenchymatous pericarp tissue which entirely fills the hilar orifice, and is fused with the inner integument at the hilar margins.

A zone of this conducting pericarp tissue lying just outside the hilar orifice and including the elements which are fused with the inner integument becomes greatly contracted radially and darkly pigmented during the maturation of the caryopsis. This pigmented zone of the pericarp and the inner integument together constitute for the caryopsis an unbroken investment which is extremely resistant to the action of Javelle water and of chromic acid and has the quality of selective permeability, though it probably does not exclude any solute entirely.

As in the above-mentioned grasses, the wide hilar opening through the inner integument in corn is filled with parenchymatous tissue which before fertilization seemed to be of a texture similar to the nucellus (fig. 5, A). As the expanded nucellus degenerates, this tissue remains active for a time and with well-marked nuclei in slowly enlarging cells appears by contrast to be densely cellular (fig. 11, A).

As the cells attain their maximum size the nuclei disappear (fig. 11, B) although the walls continue to take a gentian violet stain with diminishing intensity until, as the kernel matures, they are crushed, contracting radially into a more or less continuous and homogeneous layer which closes the hilar orifice and completes a protective circle of which the semipermeable membrane of the testa is the longer segment. The consistency, continuity, and intensity of color of this compressed layer vary with the age of the kernel and depend somewhat on the strain of corn (figs. 11, C; 12, A, B, C). In section, under the microscope, it is yellow to golden brown in color and appears to have a fatty or waxy consistency; to the naked eye, its color is dark brown in the mature grain, and its location and extent may be seen by clipping away the spongy cap of the kernel (fig. 12, C). It may be exposed with little difficulty, for, as the moisture content of the maturing kernel is lowered, the tissues of the pedicel forming the so-called "tip cap" of the shelled kernel contract and a cavity forms between the hilar layer and the vascular elements of the cap (fig. 12, B). Although this layer is more resistant to hydrolysis by 72-percent H_2SO_4 than adjoining parts, except the semipermeable membrane of the testa, it does not respond to the Sudan III color test for suberin.

MARGINAL LAYER OF ENDOSPERM

The cells in the part of the outermost layer of the endosperm that is coextensive with the testa present the appearance usually associated with the aleurone layer in cereals (fig. 13, A, B). Those at the base of the endosperm facing the hilar region, however, seem to be entirely different. They are more or less completely filled with a streaked substance quite unlike the granular contents of the aleurone cells (fig. 13, A, C, D). Dissimilarity in the nature of the cell contents of the two segments is further indicated in infected kernels. Sections show comparatively few hyphae even making their way intercellularly across the modified layer (fig. 14, C), although the aleurone cells are in most cases packed with fungus (fig. 14, D). The two sections of the marginal layer of the endosperm show their dissimilarity early; they differ also in thickness, the aleurone layer being for the most part one cell thick, while across the hilum region

marginal modification may extend inward with diminishing intensity to a depth of several cells (fig. 13, *B*, *D*). Until maturity, both are

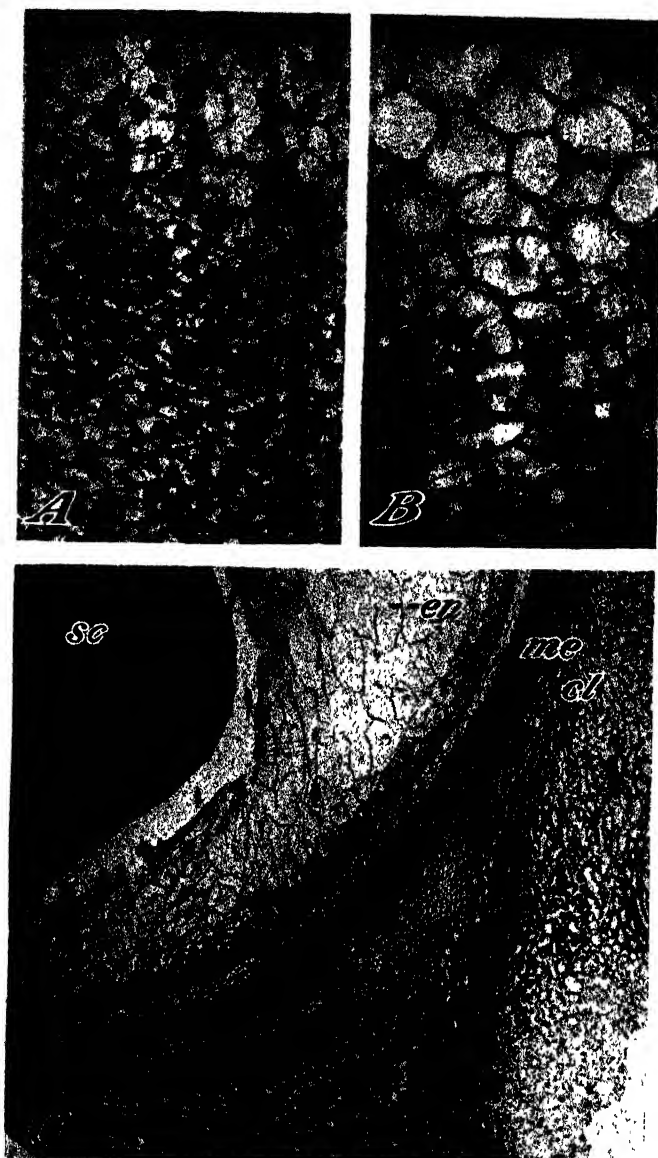


FIGURE 11.—Changes in the cells in hilar region: *A*, Fixed on August 16, 2 days after pollination. $\times 280$. *B*, open-pollinated, fixed on August 19, stained with triple stain. $\times 280$. *C*, portion of a longitudinal section of a maturing kernel of *L*, fixed 42 days after pollination, showing an early stage in the formation of the closing layer of the hilar orifice; *se*, scutellum; *ep*, endosperm; *me*, marginal layer of endosperm facing the hilar orifice; *cl*, closing layer; *c*, chalasa. Stained with triple stain. $\times 48$.

colorless under the microscope. In the mature kernel parts of the modified marginal layer lying close to the dark layer which bridges

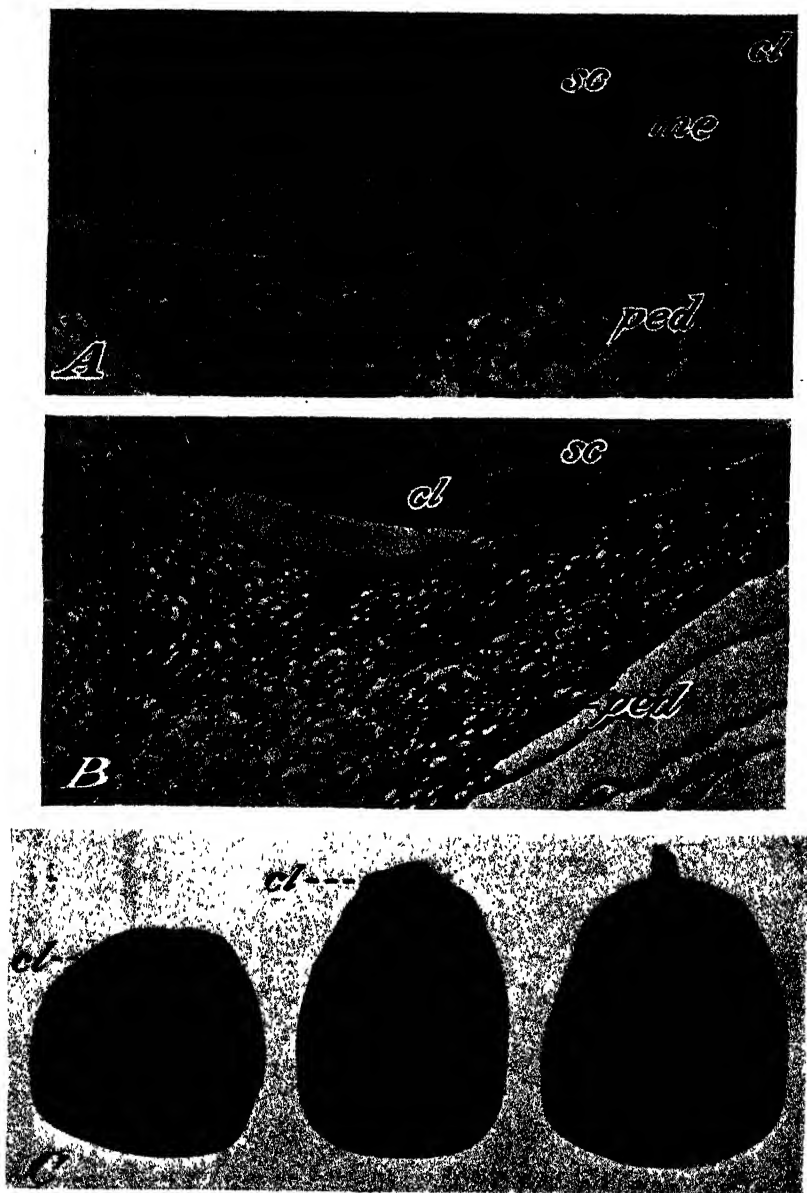


FIGURE 12.—The closing layer of the hilar orifice. A and B. Portions of longitudinal sections of maturing kernels, showing progressive stages in the formation of the closing layer of the hilar orifice; *cl*, closing layer; *sc*, scutellum; *me*, marginal layer of endosperm facing hilar orifice; *ped*, pedicel. Stained with triple stain. $\times 48$. C. Pedicel ("tip cap") removed from two kernels at left to show brown closing layer of hilar orifice (*cl*); at right, kernel with "tip cap." \times about 4.

EXPLANATORY LEGEND FOR FIGURE 13

A and C. Portions of longitudinal sections through the tip of the kernel in the chalazal region, showing the two parts of the marginal layer of the endosperm. The change in the character of the cells in this layer is abrupt rather than gradual. An arrow points to the line of demarcation in A. A. From an immature kernel of strain A48 grown at Bloomington, Ill.: *t*, Testa; *p*, pericarp; *cl*, aleurone layer; *sc*, endosperm; *c*, chalazae; *me*, marginal cells of endosperm facing the hilar orifice; *A*, parenchymatous pericarp tissue in the hilar region. Stained with triple stain. $\times 98$. B. Portion of a cross section of a maturing kernel of strain A48 grown at Madison, Wis., showing the cells of the aleurone layer: *cl*, Aleurone layer; *sc*, starchy



FIGURE 13.—Marginal layer of endosperm. (For beginning of legend see opposite page.)

endosperm; *As*, horny endosperm; *t*, testa; *p*, pericarp. Stained with triple stain. $\times 285$. C, From a dormant mature kernel of a Golden Glow strain grown at Madison, Wis. The testa is more highly suberized in this section than in A and B, as shown by its failure to stain with triple stain. *en*, Endosperm; *sm*, suberized membrane of testa; *al*, aleurone layer; *mg*, marginal layer of endosperm opposite the hilar orifice; *p*, pericarp. Stained with triple stain. $\times 465$. D, Portion of a section of the marginal layer of the endosperm of a maturing kernel of L corn, showing the character of the cells in the region of the hilar orifice; *mg*, Marginal layer of endosperm; *h*, parenchymatous tissue in the hilar orifice. Stained with triple stain. $\times 285$.

the hilar orifice often take on a yellow color. Weatherwax (12, p. 376) considers this tissue to have a placental function. He says:

Its structure and position from the first suggest a special function. Its cells are relatively small, and usually elongated and angular in shape. The nuclei and dense cytoplasm have the appearance of an active living condition, and karyokinetic figures are frequent. It is also in the position nearest the vascular complex which supplies the ovule. These facts indicate that this specialized region has a placental function, taking food from the source of supply and passing it on to other parts of the endosperm. This theory is further supported by the position of this tissue relative to the order of deposition of food in the endosperm.

PERICARP

In the course of development the wall of the ovary becomes the pericarp of the caryopsis. At flowering time the ovary wall consists of a parenchymatous tissue of considerable thickness with a well-defined epidermal layer on the outer surface and a more delicate one on the inner wall.

Some sections show that shortly after degenerative changes appear in the integuments deterioration occurs also in the adjacent layers of the ovary wall (fig. 15, A), so that when, a few days after fertilization, these parts have come in contact with one another, it is difficult to identify the limits of each. In other cases, the cells of the inner epidermis are seen to maintain their identity, although they have become more or less separated by the rapid enlargement of the ovule which they had encircled (fig. 10, A). As development of the kernel continues, the cells near the base of the ovary and on the inner side of the ovary wall become more spongy in character, while the cell walls of the outer layers thicken, show numerous pits, and respond to safranin stain. Most of the spongy cells of the pericarp, with the exception of those near the base of the kernel, gradually disappear, so that in the mature caryopsis the larger part of the pericarp is composed of thick-walled pitted cells which usually become quite compact over the distal surfaces. Over the coleoptile region of the embryo both types of cells persist in considerable numbers, while in the area to be ruptured by the emerging coleorhiza only the thin-walled parenchyma is found (fig. 15, B). A relatively thin layer of cutin covers the outer surface of the pericarp.

Beeskow⁷ has reported finding traces of suberin in the pericarp of a strain of Hickory King corn with which he was working. Its presence in any considerable quantity in the pericarp, tip cap, or rachilla might possibly be an important factor contributing to resistance to fungus invasion. In the sections examined by the writer, however, no barriers of this nature were demonstrated in these regions, although the presence of the thin suberized membrane of the testa was clearly shown.

When the hull is peeled from a soaked kernel, cleavage is along the line of least resistance. In the mature corn kernel this is in the spongy inner layer of the pericarp, outside the circle of the semipermeable membrane of the testa and the closing layer of the hilar orifice (fig. 10). The removable hull thus consists of pericarp tissue and does not include the suberized semipermeable membrane of the testa.

⁷ BEESKOW, H. C. THE SELECTIVE SEMIPERMEABILITY OF THE SEED COAT OF CORN. (Unpublished master's thesis, Univ. Chicago.) 1924.

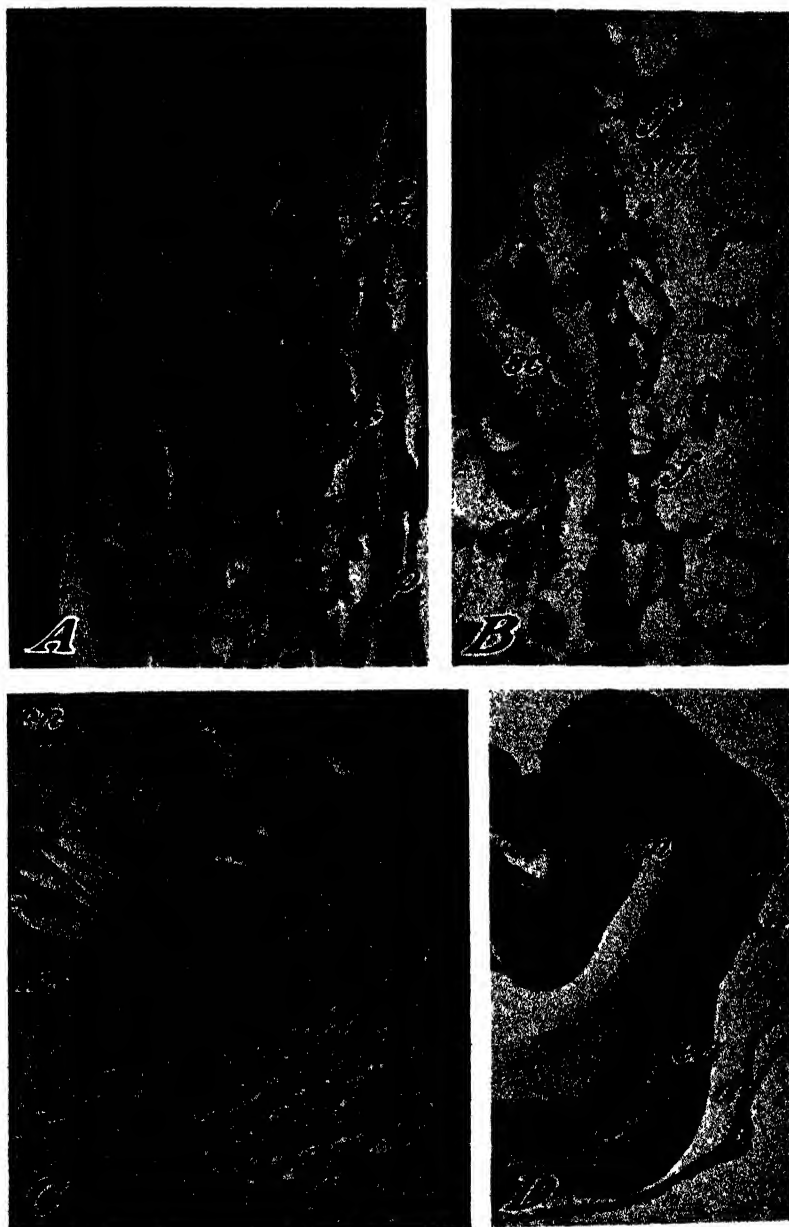


FIGURE 14.—Sections from kernels infected with *Diplodia*. A and B, Longitudinal sections, showing portions of the suberized membrane of the testa over the embryo. The membrane exhibits considerable resistance to fungal penetration and preserves its identity after the walls of neighboring infected cells have almost disappeared. *sc*, Scutellum; *em*, suberized membrane; *j*, fungus hyphae; *p*, pericarp. Stained with Delafield's haematoxylin, safranin, and orange G. $\times 430$. C, Portion of a longitudinal section through the hilum region of an infected immature kernel. The closing layer has not been formed and hyphae have advanced from the pedicel into the endosperm. *em*, Endosperm; *me*, marginal layer of endosperm facing the hilar orifice. Stained with Delafield's haematoxylin, safranin, and orange G. $\times 160$. D, Part of a cross section of a badly infected kernel. Hyphae have invaded all tissues and are massed in the aleurone cells. *al*, Aleurone layer; *em*, embryo; *p*, pericarp; *en*, endosperm; *d*, *Diplodia* pyrenidia. $\times 17$.

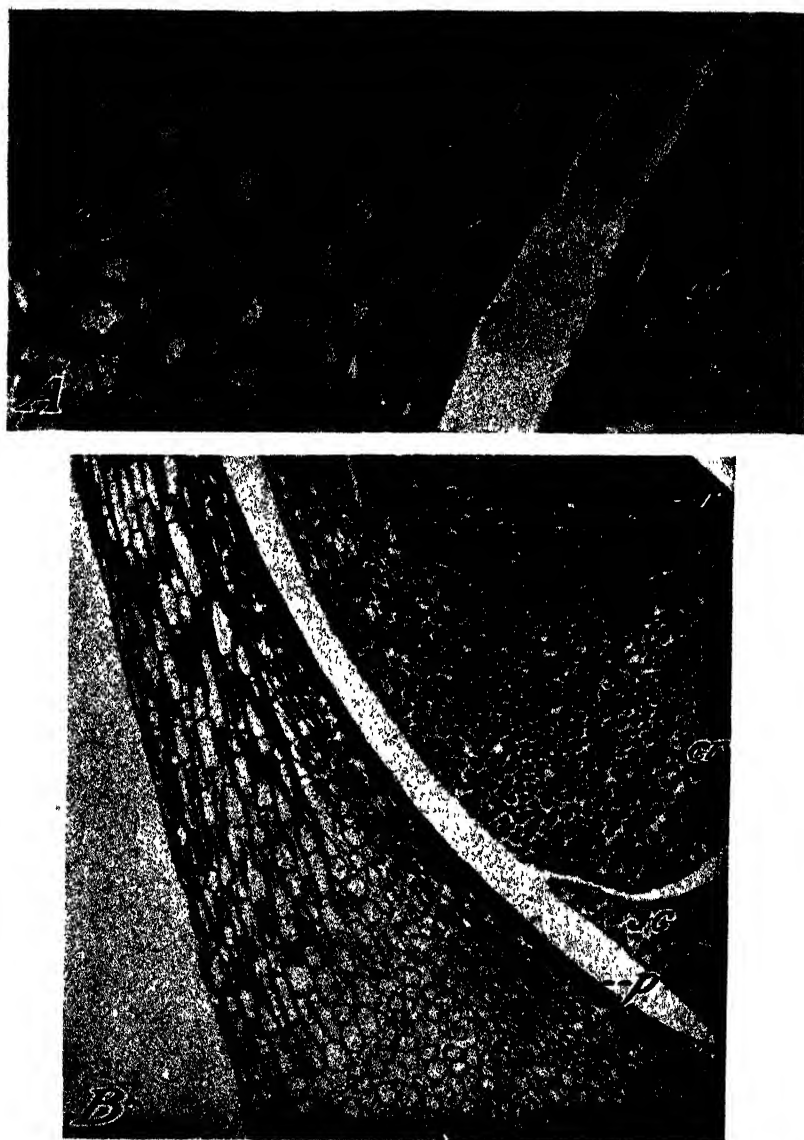


FIGURE 15.—Sections of the pericarp at different stages of development. *A*, Portion of a longitudinal section of a developing kernel fixed shortly after fertilization, showing degenerative changes beginning in the inner layers of the pericarp and integuments. There is an abundance of starch in the cells of the pericarp. *ep*, *ep*, Epidermis of nucellus; *ti*, inner integument; *oi*, outer integument; *p*, pericarp; *s*, starch. Stained with triple stain. $\times 430$. *B*, Portion of a longitudinal section of a maturing kernel, showing the spongy structure of the pericarp in the region to be ruptured during germination by the emerging coleorhiza: *r*, Root tip; *cr*, coleorhiza; *sc*, scutellum; *p*, pericarp. Stained with triple stain. $\times 58$.

DISCUSSION

Examination of the varied and representative material from pedigreed strains of yellow dent corn grown at Bloomington, Ill., and Madison, Wis., provided by collection of specimens at intervals during the summers of 1930-33 failed to show the presence of anatomical differences sufficient in themselves to account for high degrees of resistance or susceptibility to diplodia kernel rot manifested in the field (figs. 16, 17). However, variations which may have a bearing on the problem were observed (1) in the speed with which the hilar orifice is closed, (2) in the thickness and compactness of the closing layer, and (3) in the completeness of the union of the closing layer with the ends of the suberized membrane of the testa. The brown closing layer of the hilar orifice is, at its best, relatively thick and impervious to fungi and when joined to the ends of the suberized semipermeable membrane of the testa blocks advance into the seed in that region. If, however, formation of the hilar layer is delayed or incomplete, hyphae may pass around the ends of the testa and enter the embryo by way of the hilar orifice (fig. 18, A, B, C). From the material examined it may be said in general that it was in those strains known to be most susceptible in the field that a delayed and less effective closing of the hilar orifice was found. Not only is the presence of an effective closing layer in the hilar orifice an actual barrier to fungus advance through that particular region of the kernel, but it seems possible also that it should be considered as associated with certain phases of the maturation process of the caryopsis. In that case, the early appearance of the closing layer, in addition to imposing an efficient physical barrier to hyphal advance, may also be indicative of chemical changes which afford a less favorable nutrient medium for fungus growth within the plant.

In cereals the suberized semipermeable membrane of the testa functions as a more or less effective barrier to fungus advance. In the case of wheat the three-layered testa is considered to be the part of the kernel most resistant to penetration by *Gibberella saubinetii* (Mont.) Sacc., becoming increasingly resistant as the kernel matures (?). The outer suberized layer is the most resistant part of the testa. It varies in thickness and its resistance is seemingly proportional to its thickness. In section, it is sufficiently broad to show distinct differences in width in the embryo and distal regions of the kernel. In the corn kernel, however, the single suberized layer of the testa is so thin that any variations in thickness which may exist are not easily seen. Nevertheless it seems probable that it also is thinnest over the embryo and in the proximal region of the kernel, for it is in these areas that its penetration by fungus occurs most frequently. Though it appears so delicate, the membrane is relatively resistant to hyphal penetration and maintains its identity surprisingly well despite the fact that no instances were observed in which hyphae massed in the adjacent pericarp failed to pierce it (fig. 14, A, B). Once within the circle of the testa, fungus growth in the aleurone layer is rapid. These cells seemingly present a very favorable medium for *Diplodia* and soon become packed with hyphae. It is in this region also that most of the pycnidia in the kernel may be found (figs. 14, C; 19).

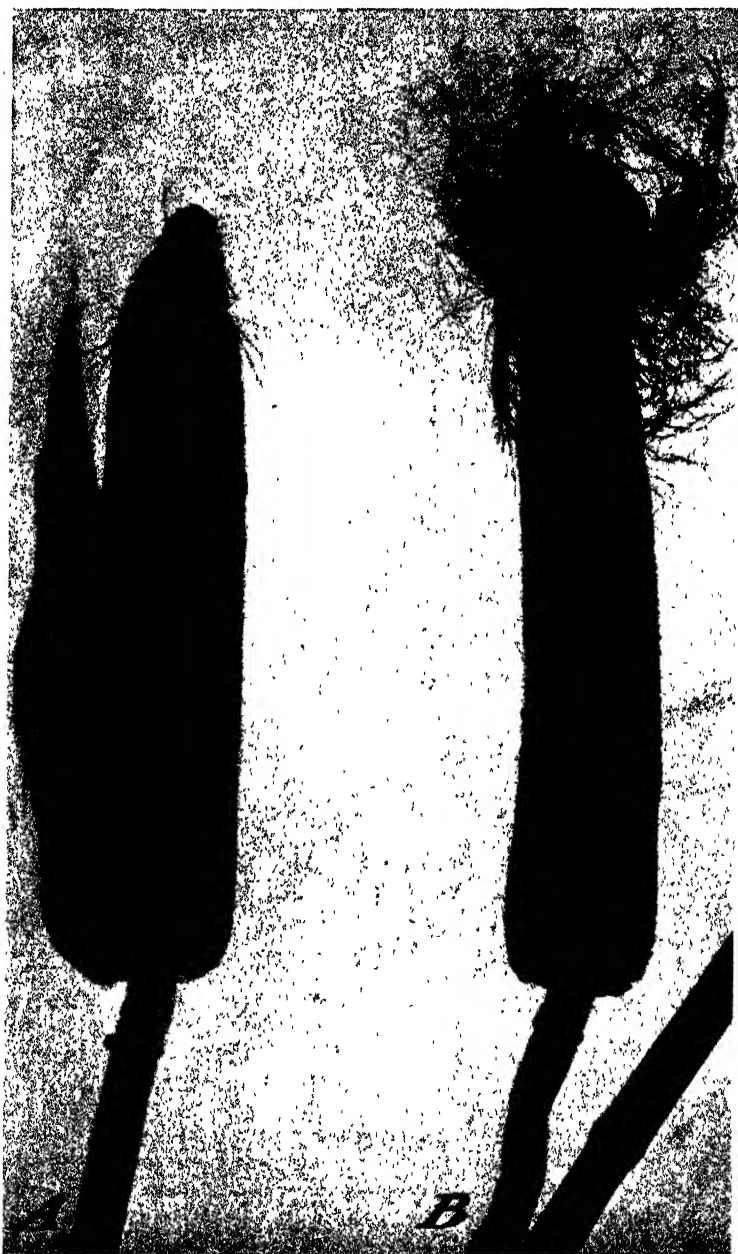


FIGURE 16.—Ears of cross 58, a strain of yellow dent corn that has proved resistant to *Diplodia* ear rot in the field. Inoculated by a hypodermic injection of a spore suspension of *Diplodia zeae* on August 26; harvested September 12 at Bloomington, Ill. A, Tip inoculated; B, shank inoculated.



FIGURE 17.—Ears of Krug X Lan, a strain of yellow dent corn that has proved susceptible to *Diplodia* ear rot in the field. Inoculated hypodermically with a spore suspension of *Diplodia zeae* on August 25 and harvested September 12 at Bloomington, Ill. A, Shank inoculated; B, tip inoculated.

During the development of the kernel, changes in the carbohydrate content of the endosperm occur which may also have a bearing on the host-fungus relationship in that region. In her microchemical studies of the developing endosperm of maize, Lampe (5) reports finding

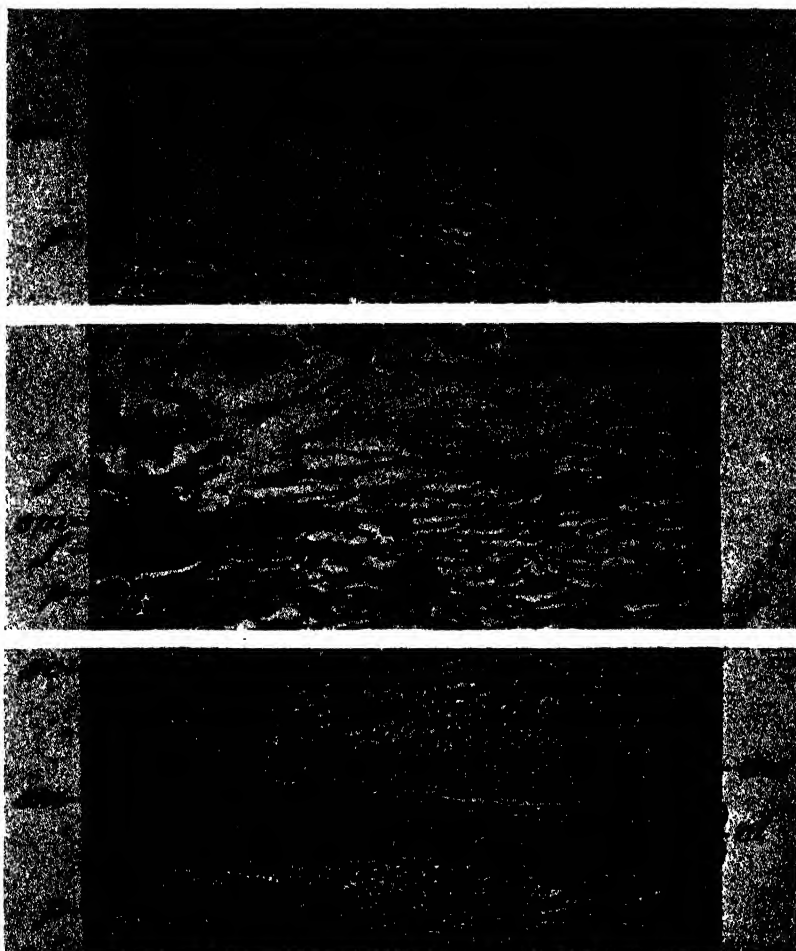


FIGURE 18.—Detail sections of kernels from ears shown in figures 16 and 17. A, Portion of a longitudinal section of an infected kernel of the Krug x Lan strain harvested September 12 (fig. 17), showing the end of the suberized membrane of the testa and the absence of the closing layer in the hilar orifice: *me*, Marginal layer of endosperm facing the hilar orifice; *sm*, suberized membrane of testa; *p*, pericarp. Stained with Sudan III, mounted in glycerin. $\times 335$. B, Section from same kernel as A, stained with Delafield's haematoxylin, safranin, and orange G to show the presence of hyphae in the hilar orifice and on both sides of the suberized membrane: *f*, Fungus hyphae; *sm*, suberized membrane; *p*, pericarp. $\times 335$. C, Portion of a longitudinal section of a kernel of cross 58 harvested on September 12 (fig. 16), showing the presence of the closing layer of the hilar orifice and its close contact with the end of the suberized membrane of the testa. The contrast of the natural yellow color of the closing layer with the hyaline cells on either side is lost in the photomicrograph. *sc*, Scutellum; *sm*, suberized membrane; *p*, pericarp; *me*, marginal layer of endosperm; *cl*, closing layer. Stained with Sudan III, mounted in glycerin. $\times 335$.

reducing sugars present during the early development in all types of corn. In older kernels polysaccharides were found in the distal part and reducing sugars at the base. As the region containing polysaccharides increased, that containing reducing sugars decreased, until

at maturity, in nonsweet corn, the disappearance of sugars was essentially complete.

Infection in the kernel appears usually to occur at the proximal end of the caryopsis. Hyphae were observed to enter the pedicel near the bases of the glumes or to have entered from the cob. This accords with Branstetter's (2, p. 17) findings as to the location of fungi in infected kernels shown by his platings. He states:

These results seem to warrant the assumption that if a corn grain is infected, the tip one-fifth invariably contains the fungus, since in no case when the tip failed to show infection did any other part of the kernel show infection.



FIGURE 19.—Detail of figure 14, *D.* Pycnidia of *Diplodia zeae*, containing mature spores, located along the aleurone layer in a badly infected kernel. Stained with Delafield's haematoxylin, safranin, and orange G. $\times 220$.

In a large percentage of diplodia inoculations in the field under conditions favorable for infection, hyphae were found in the spongy tissues of the pedicel and the proximal pericarp of the kernel. In some cases these regions supported an abundant fungus growth; in other strains a few straggling hyphae were visible. Since no anatomical differences sufficient to account for the two conditions were apparent in the hosts, it seems not unreasonable to suppose that such lack of uniformity in the host-fungus relationship may perhaps be tied up more closely with the chemical medium offered by the host in its relation to the nutritional requirements of the parasite than with anatomical differences in kernel structure. Further experiments along this line have been undertaken.

SUMMARY

This paper outlines briefly the development and microscopic anatomy of the caryopsis of dent corn and is concerned primarily with those structures which might have a bearing on the problem of host resistance and susceptibility to fungus diseases in general and to invasion by *Diplodia zeae* (Schw.) Lév. in particular.

Unless otherwise noted, the specimens examined were from hand-pollinated ears of yellow dent corn grown at Bloomington, Ill., and Madison, Wis. During the early periods of development of the kernel, collections were made at intervals varying from 2 to 7 days.

Sections of the ovary showed that the outer integument covers all but a relatively narrow triangle of the surface of the inner integument and that the outer and inner integuments degenerate at about the same time, leaving a testa consisting of a single very thin suberized semipermeable membrane, which originated along the inner wall of the inner integument in close contact with the epidermis of the nucellus. It was not determined whether the entire marginal wall of the integument had been impregnated with suberin or whether a layer of suberin had been laid down along its surface as is the case in the wheat kernel. The presence of the membrane was not demonstrated as long as the inner integument and the epidermis of the nucellus were separable.

The two sections of the marginal layer of the endosperm are pictured. The formation of the closing layer of the hilar orifice is shown, and attention is called to the possible relation of the variations found in this region to fungus advance into the embryo. Although these points may have a bearing on the problem of resistance or susceptibility to kernel rots, no anatomical features were observed sufficient in themselves to account for the differences in resistance or susceptibility shown by various strains of corn in the field.

Diplodia infection in the kernel was found to begin in almost every case at the proximal end of the kernel. In some strains the spongy tissues of the pedicel and proximal pericarp were filled with the fungus; in others, only a few straggling hyphae could be found in those regions. Anatomical features sufficient in themselves to account for such differences did not appear to be present. It seems, therefore, not unreasonable to conclude that such lack of uniformity in the host-fungus relationship may perhaps be tied up more closely with the chemical medium offered by the host in its relation to the nutritional requirements of the parasite than with anatomical differences in kernel structure.

LITERATURE CITED

- (1) ANDERSON, A. M.
1927. DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND CARYOPSIS OF *POA PRATENSIS* AND *POA COMPRESSA*. Jour. Agr. Research 34: 1001-1018, illus.
- (2) BRANSTETTER, B. B.
1927. CORN ROOT ROT STUDIES. Mo. Agr. Expt. Sta. Research Bull. 113, 80 pp., illus.
- (3) HADDAD, E. S.
1931. MORPHOLOGICAL DEVELOPMENT OF SWEET CORN PERICARP IN TWO INBRED LINES AND THEIR F_1 HYBRID. Ind. Agr. Expt. Sta. Bull. 347, 24 pp., illus.
- (4) HARRINGTON, G. T., and CROCKER, W.
1923. STRUCTURE, PHYSICAL CHARACTERISTICS, AND COMPOSITION OF THE PERICARP AND INTEGUMENT OF JOHNSON GRASS SEED IN RELATION TO ITS PHYSIOLOGY. Jour. Agr. Research 23: 193-222, illus.

-
- (5) LAMPE, L.
1931. A MICROCHEMICAL AND MORPHOLOGICAL STUDY OF THE DEVELOPING
ENDOSPERM OF MAIZE. *Bot. Gaz.* 91: 337-376, illus.
- (6) MILLER, E. C.
1919. DEVELOPMENT OF THE PISTILLATE SPIKELET AND FERTILIZATION IN
ZEA MAYS L. *Jour. Agr. Research* 18: 255-266, illus.
- (7) PUGH, G. W., JOHANN, H., and DICKSON, J. G.
1932. RELATION OF THE SEMIPERMEABLE MEMBRANES OF THE WHEAT
KERNEL TO INFECTION BY GIBBERELLA SAUBINETII. *Jour. Agr.*
Research 45: 609-626, illus.
- (8) RANDOLPH, F. R.
1926. A CYTOLOGICAL STUDY OF TWO TYPES OF VARIEGATED PERICARP IN
MAIZE. N. Y. (Cornell) *Agr. Expt. Sta. Mem.* 102, 14 pp., illus.
- (9) ROBBINS, W. W.
1931. THE BOTANY OF CROP PLANTS, A TEXT AND REFERENCE BOOK.
Ed. 3, rev., 639 pp., illus. Philadelphia.
- (10) TRUE, R. H.
1893. ON THE DEVELOPMENT OF THE CARYOPSIS. *Bot. Gaz.* 18: 212-226,
illus.
- (11) WEATHERWAX, P.
1917. THE DEVELOPMENT OF THE SPIKELETS OF ZEA MAYS. *Bull. Torrey*
Bot. Club 44: 483-496, illus.
- (12) ————
1930. THE ENDOSPERM OF ZEA AND COIX. *Amer. Jour. Bot.* 17: 371-380,
illus.

INFLUENCE OF WEATHER FACTORS ON MOISTURE CONTENT OF LIGHT FUELS IN FORESTS OF THE NORTHERN ROCKY MOUNTAINS¹

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INTRODUCTION

The necessity of forest-fire protection is generally recognized in the United States. The tremendous damage done by forest fires each year to valuable timber, watershed cover, forest range, wildlife, recreational facilities, and personal property has impressed upon the people the need for preventing and controlling forest fires so far as this is humanly possible.

In the forests of northern Idaho and of Montana the fire-protection problem is particularly difficult, owing to the inflammable character of the forest vegetation and to the dryness of the summer weather. On the 23 million acres of national-forest land in this area, during the 10-year period 1925-34 there occurred a yearly average of 1,356 fires, which burned over 114,000 acres, caused damage totaling nearly three-quarters of a million dollars, and required expenditure of a similar amount for control.

Fire-control experience has shown that in this northern Rocky Mountain region approximately 50 percent of the area burned and 35 percent of the suppression costs result from only 1 percent of the fires.

Obviously, in this region satisfactory fire control can be attained only if the fire-protection organization succeeds in suppressing fires while they are still small. In efforts toward this end, administrative officers are aided by knowledge of current forest-fire danger.³ A complete understanding of fire danger from day to day is difficult to obtain because many factors are involved, including season of year, activity of fire-starting agencies, topography, character of green vegetation, weather, fuel type, fuel volume, and fuel moisture content. By making observations or measurements of several of the most important factors and properly integrating them, however, the forest protectionist can determine the relative fire danger existing at a given time and place. In this way he can learn whether his force should be temporarily expanded or reduced, and how it should be distributed over the area for which he is responsible. In addition, through a knowledge of the influence of given factors on fire danger the protectionist is assisted in interpreting weather forecasts and thus in preparing for all classes of fire danger that are likely to arise.

¹ Received for publication July 29, 1935; issued February 1936.

² The writer is indebted to F. K. Schumacher, of the Division of Silvical Research, Forest Service, for guidance in planning the statistical analysis involved in this study.

³ "Forest fire danger" as used here is a general term expressing the sum total of the factors that determine whether fires will start, spread, and do damage.

SIGNIFICANCE OF MOISTURE CONTENT OF FOREST FUELS

Fuel-moisture content alone serves as a criterion of forest-fire danger. The wetness or dryness of forest fuels largely determines their inflammability or ease of ignition and the rate of spread of forest fire. Obviously, each fuel must be heated to a certain temperature before combustion results, and anything that retards this heating reduces fire danger. Water in the fuels absorbs heat before it is driven off in the form of vapor; consequently it delays the raising of fuel temperatures to the kindling point. Knowledge of the relation of the moisture content of the common forest fuels to their inflammability, or to the rate of spread of fire, or to both, is essential in determining current fire danger.

The moisture content of lightweight fuels such as duff and small branch wood is particularly important because of their wide-spread distribution in almost all forest types and because they serve as carriers of fire from tree to tree and from log to log. Duff commonly composes a greater part of the surface layer of the forest floor than any other single fuel.

Show (9),⁴ Larsen (7), Stickel (10, 11), and Gisborne (2) proved the important relation of moisture content to the inflammability of duff⁵ by making burning tests. The latter two workers tested in detail the ease of igniting duff of given moisture content with different firebrands. Gisborne, working with undisturbed duff in western white pine (*Pinus monticola*) forests in northern Idaho, concluded that whenever the duff has less than 10 percent moisture content it can be ignited by any firebrand producing heat equal to that of an ordinary match, that at more than 13 percent moisture the duff is generally immune to ignition by burning matches, and that camp fires can ignite the duff and cause fire to spread through it whenever it contains less than 18 percent moisture. On the basis of these findings and of general observation, Gisborne defined the following six degrees of susceptibility of the top layer of duff to ignition by various common firebrands:

Degree of susceptibility:	Moisture content (percent)
Noninflammable.....	More than 25.
Very low inflammability.....	25 to 19.
Low inflammability.....	18 to 14.
Medium inflammability.....	13 to 11.
High inflammability.....	10 to 8.
Extreme inflammability.....	7 to 0.

Stickel (10) made a detailed investigation in the Adirondack Mountains to determine the moisture content necessary to prevent ignition of duff by matches, cigarette butts, pipe heels, locomotive sparks, and small camp fires. His results, from 370 tests, are summarized in table 1.

⁴ Reference is made by number (italic) to Literature Cited, p. 905.

⁵ In this discussion the term "duff" applies to the surface layer of the forest floor, that is, to the "litter", which is made up of vegetable-matter deposits only slightly decomposed.

TABLE 1.—*Surface duff-moisture contents at which various firebrands are effective*¹

Degree of hazard ²	Surface duff moisture content, percent	Firebrands effective
Extreme.....	Less than 6.....	Cigarettes, locomotive sparks, pipe heels, matches, and camp fires.
High.....	6 to 10.....	Locomotive sparks, pipe heels, matches, and camp fires.
Medium.....	11 to 16.....	Pipe heels, matches, and camp fires.
Low.....	17 to 22.....	Matches and camp fires.
Very low.....	23 to 29.....	Camp fires. ³
Generally safe.....	30 or more.....	None.

¹ According to findings of Stikel (10).² "Hazard", in Stikel's classification, has the same meaning as is attached in this paper to "forest-fire danger" (see footnote 3, p. 885).³ Duff at edge will smolder, but fire will not spread much.

Rate of spread of forest fire has definitely been shown to depend principally upon fuel-moisture content. The Quartz Creek fire of 1926 (2) and the Freeman Lake fire of 1931 (4), both in northern Idaho, made their longest runs on days when duff moisture was exceptionally low. The latter fire, which spread at an average rate of 1,600 acres per hour for 12½ hours, began at a time when the duff-moisture content was about 5 percent. In no recorded instance has a "blow-up" occurred when the moisture content of lightweight fuels was relatively high.

DETERMINANTS OF FUEL MOISTURE CONTENT

The several weather elements, acting together, are the most important determinants of forest fuel moisture content. When the weather is wet, the fuels are wet or tend to become so; and dry weather produces dry conditions in the fuels. The changes, of course, require more or less time, the lag depending upon how wet or dry the fuels were to begin with. In the northern Rocky Mountains, after a heavy rain a week or more of hot, drying weather may be required to produce extreme danger.

The moisture content of light fuels such as duff and small branch wood bears an especially close relation to current weather. Duff, in particular, because of its loose distribution and porous structure, picks up or loses moisture rapidly when atmospheric conditions change.

Differences in the moisture contents of similar fuels on parts of the same area may be due in part to weather variations between one place and another, but no doubt are due principally to differences in degree of exposure of the fuels. A dense forest canopy intercepts most of the direct rays of the sun and reduces wind movement, thereby lowering temperature and evaporation and increasing humidity beneath it (5). Fuel moistures, consequently, are higher under dense canopies than they are on burned-over or clear-cut areas fully exposed to sun and wind.

METHODS AND CONDITIONS OF FIELD STUDY

The study discussed here has dealt for the most part with duff-moisture content in its relation to current weather. It has dealt also with moisture content of branch wood one-half inch in diameter in its relation to weather. These relations were observed under different forest-canopy conditions.

In this study duff-moisture measurements have been confined to the uppermost $\frac{1}{2}$ -inch layer, because when the moisture content of this layer is low fire may spread through it rapidly even if the average moisture content of the entire forest floor is high.

Measurements of weather factors and fuel moisture have been made on three exposures at the Priest River branch of the Northern Rocky Mountain Forest and Range Experiment Station, in northern Idaho. Beginning in 1924, observations were taken on an area from which all trees had been removed; on an area where timber cutting had reduced the forest canopy by about half, the principal tree species of the remaining stand being western hemlock (*Tsuga heterophylla*); and in a dense stand of western white pine near the base of

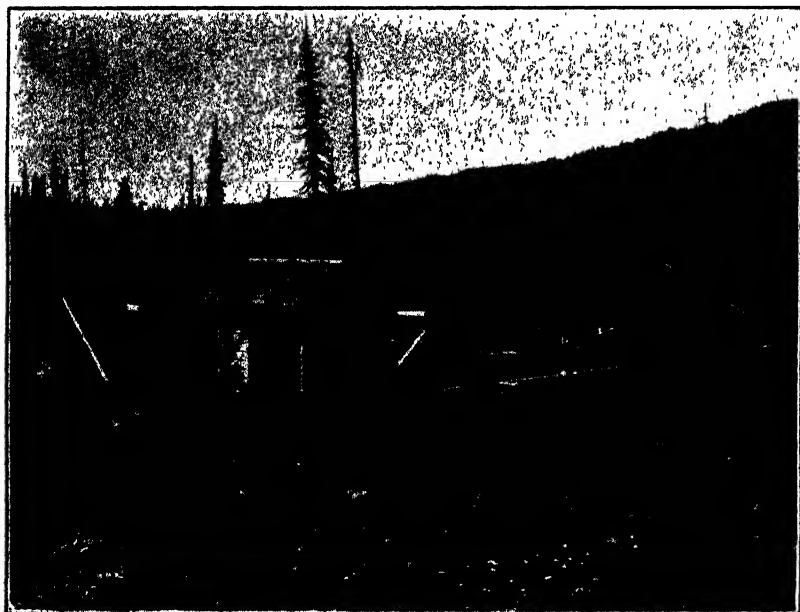


FIGURE 1.—Clear-cut station, fully exposed to weather.

a northwest slope. These areas were approximately three-quarters of a mile apart. In 1930 the original half-cut and full-timbered stations were abandoned, and new ones established on a bench within 1,400 feet of the clear-cut station. On the new half-cut station, the residual stand is similar in composition to that on the one abandoned. The new full-timbered station is occupied by the climax western white pine type, in which western hemlock and western red cedar (*Thuja plicata*) predominate. Comparative light values taken at the instrument exposures by Haig⁶ are shown in table 2. The clear-cut station, and the half-cut and full-timbered stations in use from 1930, are shown in figures 1, 2, and 3, respectively.

⁶ HAIG, I. T. CERTAIN FACTORS CONTROLLING INITIAL SEEDLING ESTABLISHMENT IN WESTERN WHITE PINE STANDS. 1935. (Unpublished doctor's dissertation. Copies on file at Yale Univ. School of Forestry, New Haven, Conn., and Northern Rocky Mountain Forest and Range Expt. Sta., Missoula, Mont.)

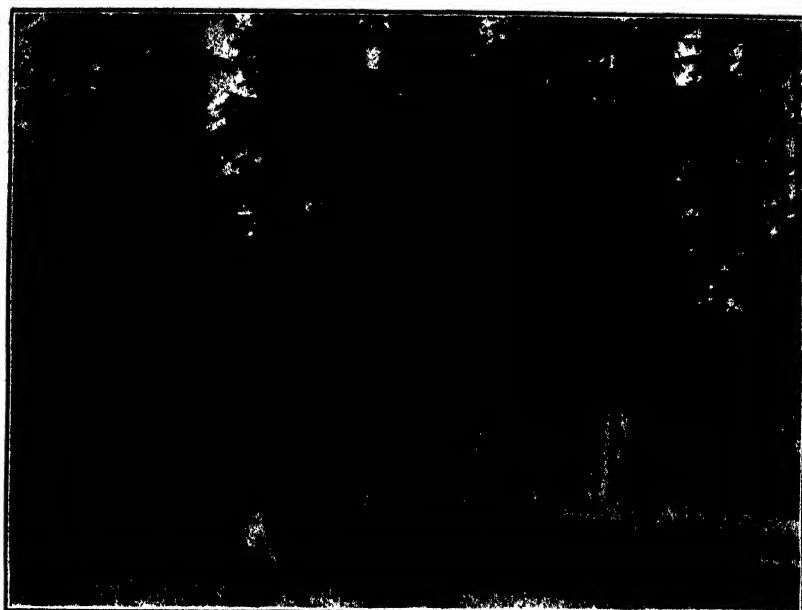


FIGURE 2.—Half-cut station; residual stand is composed chiefly of western hemlock.



FIGURE 3.—Full-timbered station; the dense virgin stand is composed chiefly of western hemlock and western red cedar.

TABLE 2.—*Light values on half-cut and full-timbered areas in use from 1930, as determined with different instruments*

Instrument	Light, in terms of full sunlight, on—	
	Half-cut area	Full-timbered area
	Percent	Percent
Clements photometer.....	24	4
Shirley thermopile.....	23	3
Livingston black and white spheres.....	26	5
Average.....	24.3	4

Careful study revealed that the old and new stations were nearly enough alike to justify joint analysis of records obtained before and after 1930.

Simultaneous measurements of weather factors and duff-moisture content on the clear-cut, half-cut, and full-timbered areas were begun in June 1924. They were made daily at 4:30 p. m. Air temperature, relative humidity, precipitation, evaporation, and temperature of the dew point were the weather elements measured in 1924. Other elements were added later. Table 3 lists the factors investigated and shows in what years measurement of each factor was begun on the three areas, respectively. Beginning with 1925, twice-daily measurements were made at all stations throughout the fire season, at approximately 9 a. m. and 4:30 p. m. These two hours were selected in order to sample extremes, preliminary investigation having indicated that fuel moisture normally was greatest between 7 and 9 a. m. and least at about 4:30 p. m. The dates on which measurements were begun and ended each year were not fixed. In general, measurements were begun in April or May, before the fire season actually started, and were terminated in late September or early October after the fire season definitely came to an end.

TABLE 3.—*Fuel-moisture and weather factors studied, and years in which measurement of each was begun on the three study areas, respectively*

Factor	Year in which measurements were begun on—		
	Clear-cut area	Half-cut area	Full-timbered area
Fuel moisture content:			
Duff moisture.....	1924	1924	1924
1/4-inch wood moisture.....	1929	1929	1929
Weather elements and associated factors:			
Maximum air temperature.....	1924	1930	1930
Current air temperature.....	1924	1924	1924
Minimum air temperature.....	1924	1930	1930
Maximum duff temperature.....	1930	1930	1930
Temperature of the dew point.....	1924	1924	1924
Current relative humidity.....	1924	1924	1924
Minimum relative humidity.....	1924	1930	1930
Average relative humidity.....	1924	1930	1930
Wind.....	1930	1930	1930
Precipitation.....	1924	1930	1930
Evaporation.....	1924		
Number of days since 0.01 inch of precipitation.....	1924	1924	1924
Number of days since .18 inch of precipitation.....	1924	1924	1924
Number of days since .20 inch of precipitation.....	1924	1924	1924
Number of days since .30 inch of precipitation.....	1924	1924	1924
Number of days since .40 inch of precipitation.....	1924	1924	1924

ANALYSIS OF DATA, AND APPLICATION OF RESULTS OF ANALYSIS

Gross correlations indicate the forest-fuel moistures that normally exist at given air temperatures and humidities; they fail, however, to show the relative or net influence of temperature or of humidity on fuel-moisture content. None of the weather elements acts independently of the others. Hence, in order to determine fundamental relations between fuel-moisture content and the weather it was necessary to subject weather and fuel-moisture data to multiple-correlation analysis.

METHOD OF ELIMINATING CURVILINEARITY

A preliminary multiple-correlation study involving duff moisture as the dependent variable and several weather elements as the independent variables produced, in all cases, curvilinear regressions.

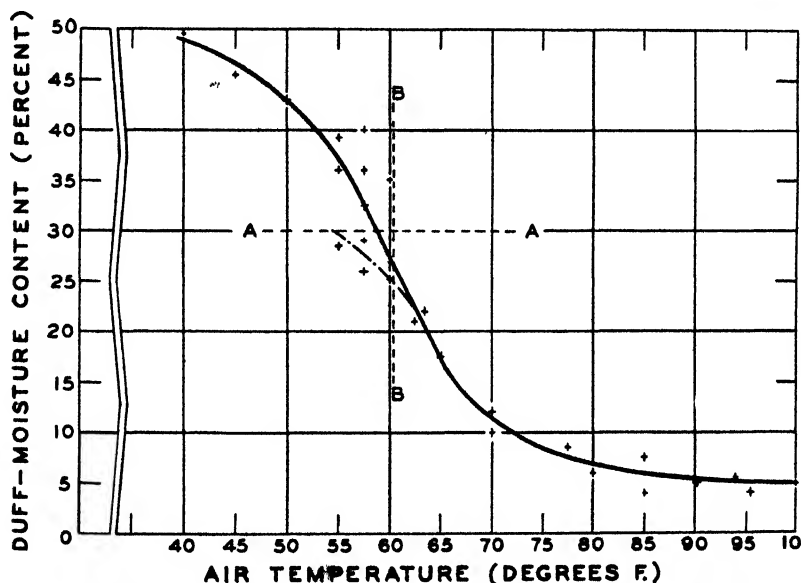


FIGURE 4.—Hypothetical regression of duff-moisture content on air temperature.

In multiple-correlation problems it is desirable to obtain algebraic expression of regressions whenever possible, because they are the simplest to compare and interpret and are not susceptible to the personal error associated with fitting freehand curves. The curvilinearity of these regressions was due to the fact that the instrument used to measure duff moistures does not show values greater than 50 percent. Direct elimination of observations of duff-moisture content amounting to, say, 30 percent or more inevitably lessens the average slope of the regression curve. These data can be eliminated without altering the slope, however, by placing a limit on an independent variable. An illustration of the effects of these two procedures follows: In figure 4 if all observations of duff moisture greater than 30 percent—that is, all the points above line *A*—are directly eliminated, the upper end of the line of best fit is changed from its former position to the one shown by the dash-dot line. T. J. M.

itation of the dependent variable, then, has changed the slope of the regression curve. However, if all observations made when air temperature was 60° F. or lower—that is, all points to the left of line *B*—are rejected all duff moistures of 30 percent or more are eliminated from the correlation without causing a change in the slope of the regression curve.

If study is restricted to observations made on the clear-cut area 2 or more days after 0.01 inch of precipitation, and on the half-cut and full-timbered areas 5 or more days and 6 or more days, respectively, after 0.01 inch of precipitation, all duff moistures of 30 percent or more are eliminated. This removes the curvilinearity in the net regressions due to restriction of the dependent variable to 50 percent. It is logical to confine the study to duff moistures of less than 30 percent, because when duff contains 30 percent or more moisture fire danger does not exist.

SELECTION OF MOST IMPORTANT VARIABLES

As is shown in table 3, data on 16 weather elements were available for analysis. This large number of variables cannot be handled conveniently by the usual correlation methods. It was thought possible that certain of these factors contribute nothing of significance to the final multiple-correlation coefficient, although each shows a definite relation to fuel moisture when correlated with it separately. For these reasons, measures were taken to select those weather elements that contributed most to the multiple correlation.

Analysis of a random sample of 130 sets of afternoon measurements for the clear-cut area according to the method developed by Kincer and Mattice (6) showed that the factors having the greatest effect on fuel moisture were current air temperature and relative humidity. Maximum duff temperature and evaporation rate were correlated almost as closely with fuel moisture as current air temperature and relative humidity, respectively. Factors having comparatively small influence on fuel moisture were temperature of the dew point, number of days since 0.01 or 0.20 inch of precipitation, and wind. These variables were retained for further investigation in preference to mean and minimum relative humidity, for example, because the latter are highly correlated with current relative humidity.

On the basis of all the available afternoon records for the clear-cut area that were made more than a day after 0.01 inch of precipitation, gross-correlation coefficients were computed for duff moisture as the dependent variable with each of the following eight factors as independent variables: Current relative humidity, current air temperature, maximum duff temperature, evaporation, temperature of the dew point, number of days since 0.01 inch of precipitation, wind, and number of days since 0.20 inch of precipitation. The results are given in table 4. Because wind and number of days since 0.20 inch of precipitation were not found to be significantly correlated with either the dependent variable, duff moisture, or with any of the other variables tested, they were dropped from consideration.

TABLE 4.—Gross-correlation coefficients¹ for all combinations of duff-moisture content (*X*) on clear-cut area and eight weather factors

Correlation coefficient <i>r</i>	Numerical value	Correlation coefficient <i>r</i>	Numerical value	Correlation coefficient <i>r</i>	Numerical value	Correlation coefficient <i>r</i>	Numerical value
<i>XA</i>	+0.660	<i>AD</i>	-0.608	<i>BH</i>	+0.103	<i>EF</i>	-0.227
<i>XB</i>	-.576	<i>AE</i>	+ .597	<i>CD</i>	+ .734	<i>EG</i>	-.160
<i>XC</i>	-.552	<i>AF</i>	+ .229	<i>CE</i>	-.053	<i>FH</i>	-.049
<i>XD</i>	-.535	<i>AG</i>	-.051	<i>CF</i>	+ .064	<i>FG</i>	-.011
<i>XE</i>	+ .312	<i>AH</i>	-.058	<i>CG</i>	-.010	<i>FH</i>	+ .161
<i>XF</i>	-.146	<i>BC</i>	+ .784	<i>CH</i>	+ .001	<i>GH</i>	-.083
<i>XG</i>	-.037	<i>BD</i>	+ .740	<i>DE</i>	-.007		
<i>XH</i>	-.028	<i>BE</i>	-.020	<i>DF</i>	+ .035		
<i>AB</i>	-.727	<i>BF</i>	+ .133	<i>DG</i>	+ .088		
<i>AC</i>	-.597	<i>BG</i>	-.121	<i>DH</i>	-.193		

¹ Based on 226 sets of afternoon observations taken 1 or more days after 0.01 inch of precipitation. Explanation of symbols: *A*=relative humidity; *B*=air temperature; *C*=maximum duff temperature; *D*=evaporation; *E*=temperature of the dew point; *F*=number of days since 0.01 inch of precipitation; *G*=wind; *H*=number of days since 0.20 inch of precipitation.

JOINT CORRELATION

When a multiple correlation of duff moisture with air temperature and relative humidity was made, a joint relation was evident. A joint relation of variables is the relation existing when the change in a dependent variable that corresponds with a change in one independent variable depends in part upon the magnitude of a second

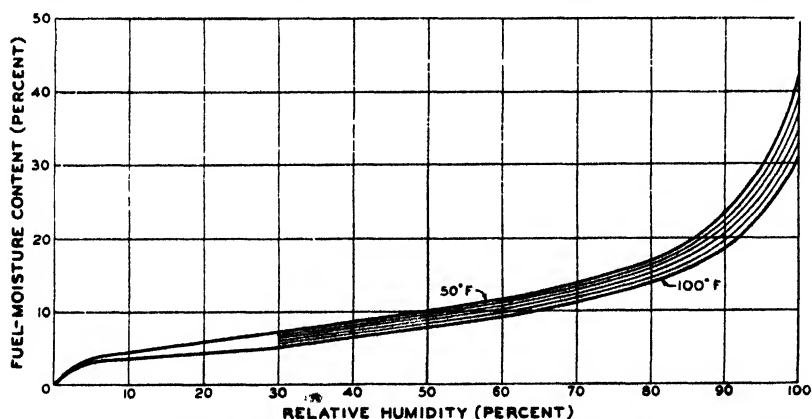


FIGURE 5.—Curve of moisture contents of coniferous duff and branch wood in equilibrium with air temperature and humidity (as determined by M. A. Dunlap).

independent variable. The equilibrium relations between fuel moisture and relative humidity at different air temperatures determined by Dunlap,⁷ of the Forest Products Laboratory, and shown in figure 5 also indicate a joint relation.

The standard linear-regression equation of the form

$$DM = aT + bRH + K \quad (1)$$

in which

DM=Duff-moisture content

T=Air temperature

RH=Relative humidity

and

a, *b*, *K*=Constants

⁷ DUNLAP, M. A. THE RELATION OF HUMIDITY TO THE MOISTURE CONTENT OF FOREST FIRE FUELS. 1924. [Unpublished manuscript. U. S. Dept. Agr., Forest Prod. Lab., Madison, Wis.]

does not show this relation. This type of equation results in parallel regression lines that do not fit the data except when all three variables are at their mean values. In other words, it represents the change in duff moisture caused by a change in relative humidity as constant regardless of air temperature. In order to reveal the true relation of air temperature and relative humidity to duff moisture, joint-correlation methods must be used.

A regression equation of the type

$$DM = a \left(\frac{RH}{T} \right) + K \quad (2)$$

was found to fit the data, as is clearly illustrated in figures 6 and 7. The lines of regression in figure 6 represent values computed separately

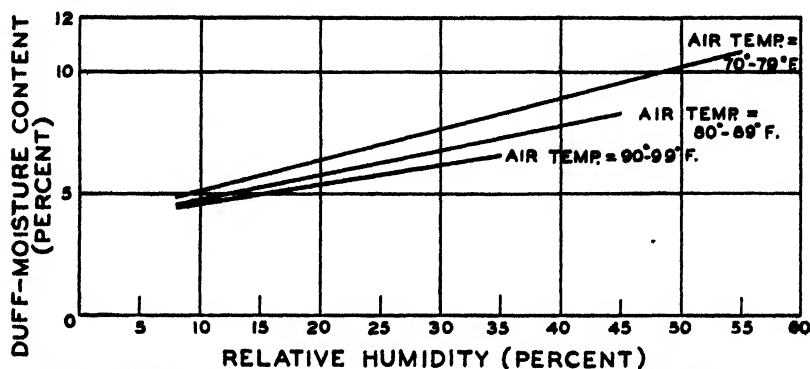


FIGURE 6.—Average actual moisture content of duff on the clear-cut area at given relative humidities and air temperatures. (Basis, 206 sets of observations taken at 4:30 p. m.)

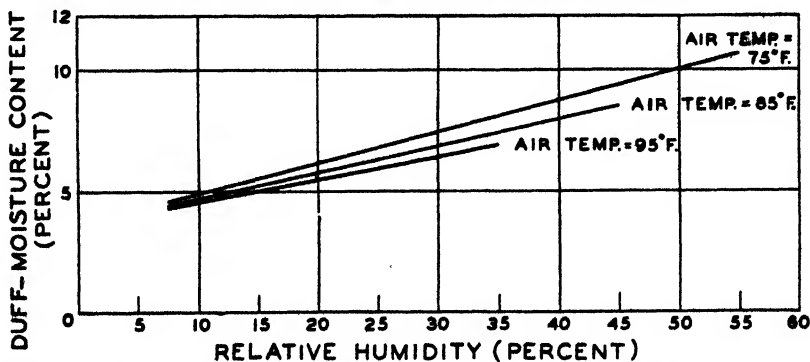


FIGURE 7.—Average moisture content of duff on the clear-cut area at given relative humidities and air temperatures, as estimated by use of the regression equation $DM = 9.64 \left(\frac{RH}{T} \right) + 3.55$. (Basis, 226 observations taken at 4:30 p. m.)

for each of the temperature classes shown; those in figure 7 were located by substituting values of temperature and humidity in equation 2.

The use of the term $\frac{RH}{T}$ is explained as follows:

Relative humidity is the ratio between the quantity of water vapor in a unit of space and the maximum quantity that unit of space can

hold, temperature and pressure remaining constant. Absolute humidity is the actual quantity of moisture in a unit of space, usually expressed in grains per cubic foot. Let

AH = Absolute humidity

AH_s = Absolute humidity at saturation.

Then, by definition $RH = \frac{AH}{AH_s}$, when temperature and pressure are constant.

But AH_s depends entirely on T . Then $RH = \frac{AH}{f(T)}$, where $f(T) =$ some function of T , or $f(T) (RH) = AH$. If $f(T) = \frac{1}{T}$, then $\frac{RH}{T} = AH$.

In other words, if $f(T)$ equals $\frac{1}{T}$, the reciprocal of temperature times relative humidity equals absolute humidity.

When the product of $f(T) \times RH$ is absolute humidity in grains per cubic foot, $f(T)$ does not vary as $\frac{1}{T}$ does, but equals the values shown in column 2 of table 5. Consequently, when $\frac{RH}{T}$ (which is the same as $\frac{1}{T} \times RH$) is equated to absolute humidity the resulting values are not in units of grains per cubic foot, or in any other units that can be identified. They are converted into grains per cubic foot, however, if multiplied by the values given in columns 3 and 6 of table 5.

Use of $\frac{RH}{T}$ as a variable was very advantageous in the correlations of this study because (1) when this term is used as a variable the regression equation actually fits the data; (2) the term is an index of absolute humidity; and (3) actual measurements of absolute humidity were not available in a form usable for the analysis, which was made by machine sorts and tabulations of punch cards. Owing to the availability of complete and lengthy records of temperature and relative humidity, great use can be made of the results of a correlation involving $\frac{RH}{T}$ as a variable. One advantage of a method of estimating fire danger through use of this variable is that it makes possible fire-danger ratings, for many stations, for past seasons during which records were made at those stations of air temperature and relative humidity but not of duff moisture.

TABLE 5.—The values of $f(T)$ in the equation $f(T) \times RH = AH$, and the values of X in the equation $X \left(\frac{RH}{T} \right) = AH$, that give absolute humidities in grains per cubic foot

Air temperature (T , °F.)	Values that give absolute humidities in grains per cubic foot		Air temperature (T , °F.)	Values that give absolute humidities in grains per cubic foot	
	$f(T)$, in the equation $f(T) \times RH = AH$	X , in the equation $X \left(\frac{RH}{T} \right) = AH$		$f(T)$, in the equation $f(T) \times RH = AH$	X , in the equation $X \left(\frac{RH}{T} \right) = AH$
50.....	0.04076	2.038	80.....	0.10934	8.747
55.....	.04849	2.667	85.....	.12736	10.826
60.....	.05745	3.447	90.....	.14790	13.311
65.....	.06782	4.408	95.....	.17124	16.268
70.....	.07980	5.586	100.....	.19766	19.766
75.....	.09356	7.017			

If $\frac{RH}{T}$ is used as an independent variable in the place of relative humidity the gross correlation with duff moisture is equally complete, as is shown by the coefficient r_{XA} in table 6, which gives gross-correlation coefficients for all combinations of duff moisture and the six weather factors having the greatest influence upon it.

TABLE 6.—Gross-correlation coefficients¹ for all combinations of duff moisture (X) on clear-cut area and the six weather factors most important in relation to it

Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value	Correlation coefficient r	Numerical value
XA	+0.672	AB	-0.778	BD	+0.740	DE	-0.007
XB	-.576	AC	-.635	BE	-.020	DF	+.035
XC	-.552	AD	-.657	BF	+.133	EF	-.227
XD	-.535	AE	+.464	CD	+.734		
XE	+.312	AF	-.210	CE	-.083		
XF	-.146	BC	+.784	CF	+.064		

¹ Based on 226 sets of afternoon observations taken 1 or more days after 0.01 inch of precipitation. Explanation of symbols: A =relative humidity divided by air temperature; B =air temperature; C =maximum duff temperature; D =evaporation; E =temperature of the dew point; F =number of days since 0.01 inch of precipitation.

GROSS AND MULTIPLE CORRELATIONS

In an endeavor to find what group of variables bear the closest relation to duff moisture, correlation coefficients were computed for all possible combinations of 2, 3, 4, 5, and 6 weather factors as independent variables with duff-moisture content as the common dependent variable. The multiple-correlation coefficients were computed from the gross r 's according to the method outlined by Wallace and Snedecor (12). They are given in table 7.

TABLE 7.—Gross- and multiple-correlation coefficients¹ for all combinations of duff moisture (X) on clear-cut area with the six weather factors most important in relation to it

Correlation coefficient <i>r</i>	Numerical value	Correlation coefficient <i>R</i>	Numerical value	Correlation coefficient <i>R</i>	Numerical value
XA.....	+0.672	X.AC.....	0.691	X.ACE.....	0.694
XB.....	-.576	X.AD.....	.683	X.ACD.....	.693
XC.....	-.552	X.AB.....	.677	X.ACF.....	.692
XD.....	-.535	X.AF.....	.672	X.ABC.....	.691
XE.....	+.312	X.AE.....	.670	X.ADE.....	.686
XF.....	-.146	X.BE.....	.650	X.ABD.....	.684
		X.CE.....	.620	X.ADF.....	.684
		X.DE.....	.617	X.ABE.....	.681
		X.BC.....	.598	X.ABF.....	.677
		X.BD.....	.598	X.AEF.....	.672
		X.CD.....	.584	X.BDE.....	.671
		X.BF.....	.580	X.BCE.....	.666
		X.CF.....	.563	X.CDE.....	.654
		X.DF.....	.550	X.BEF.....	.650
		X.EF.....	.322	X.CEF.....	.623
				X.DEF.....	.620
				X.BCD.....	.608
				X.BDF.....	.604
				X.BCF.....	.604
				X.CDF.....	.596

Correlation coefficient <i>R</i>	Numerical value	Correlation coefficient <i>R</i>	Numerical value	Correlation coefficient <i>R</i>	Numerical value
X.ACDE.....	0.697	X.ABCDE.....	0.697	X.ABCDEF.....	0.697
X.ABCE.....	.694	X.ACDEF.....	.697		
X.ACEF.....	.694	X.ABCEF.....	.694		
X.ABCD.....	.693	X.ABCDF.....	.693		
X.ACDF.....	.693	X.ABDEF.....	.690		
X.ABCF.....	.692	X.BCDEF.....	.677		
X.ABDE.....	.690				
X.ADEF.....	.686				
X.ABDF.....	.684				
X.ABEF.....	.681				
X.BCDE.....	.676				
X.BDEF.....	.671				
X.BCEF.....	.666				
X.CDEF.....	.656				
X.BCDF.....	.615				

¹ Based on 226 sets of afternoon observations taken 1 or more days after 0.01 inch of precipitation. Explanation of symbols: A=relative humidity divided by air temperature; B=air temperature; C=maximum duff temperature; D=evaporation; E=temperature of the dew point; F=number of days since 0.01 inch of precipitation.

TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN CORRELATION COEFFICIENTS

Table 7 gives evidence that the single variable $\frac{RH}{T}$, or "absolute humidity index", as it is hereafter called, is associated with duff moisture almost as closely as all six of the independent variables taken together. Evidence to this effect includes the fact that the difference between r_{XA} and $R_{X.ABCDEF}$ is only 0.025. This difference, when tested according to a method outlined by Russell (8), was found to be slightly significant. The procedure follows:

r^2_{XA} =Square of the coefficient of the correlation between duff moisture (X) and relative humidity divided by temperature (A).

$R^2_{X.ABCDEF}$ =Square of the coefficient of the multiple correlation between duff moisture (X) and six weather factors.

N=Number of observations.

Term 1= $1 - R^2_{X.ABCDEF}$

Term 2= $R^2_{X.ABCDEF} - r^2_{XA}$

If we multiply term 1 by $N\sigma^2_X$ (which is the sum of squares for variable X), the product equals the sum of squares for X independent of the part associated with variables A, B, C, D, E , and F . This sum can be considered as the unexplained sum of squares, or "error." If we multiply term 2 by $N\sigma^2_X$ the product is equal to the sum of squares for X associated with variables B, C, D, E , and F .

The test of significance consists in comparing the variance, or mean square, derived from term 2 with the variance derived from term 1. If the variance of X explained by the variables B, C, D, E , and F is significantly greater than error, then the correlation involving all seven variables is really higher than the simple correlation involving variables X and A .

When such a test of significance is made in practice, the actual values of terms 1 and 2 are used as "relative" sums of squares. Since both terms are multiplied by the same value ($N\sigma^2_X$), their relative values are not changed, and the final test of significance (table 8) is based upon the mean squares.

TABLE 8.—Computation of relative mean squares to be used in significance test

Relative sums of squares	Degrees of freedom	Relative mean squares
$1 - R^2_{X, ABCDEF}$, or 0.5142.....	$N-7$, or 219.....	0.002348
$R^2_{X, ABCDEF} - R^2_{XA}$, or 0.0342.....	5.....	.006840
$1 - R^2_{XA}$, or 0.5484.....	$N-2$	

The mean squares are obtained by dividing the relative sums of squares by the degrees of freedom associated with them, respectively.

The variance of X associated with B, C, D, E , and F can be compared with error by means of the "z test" developed by Fisher (1). The value of z is the difference between the natural logarithms of the two standard deviations; or, in terms of relative mean squares,

$$z = \frac{1}{2} \log_e \left(\frac{0.006840}{0.002348} \right) = 0.5346$$

The probability, P , of this value's being exceeded by chance is 0.015, as determined from Fisher's tables with the degrees of freedom shown in table 8. (As is shown by Fisher's tables, $z=0.3974$ and 0.5522 when $P=0.05$ and 0.01, respectively.)

According to ordinary statistical practice, P must be 0.05 or less if two variances are to be considered significantly different. In more refined tests of significance, P must be 0.01 or less. In this comparison the odds are slightly greater than 1 to 100 that the observed difference is due to chance. While the influence of variables B to F is probably significant, the inclusion of these variables for the sake of a further reduction in the remaining variance of about 3 percent is impractical.

ESTIMATES OF DUFF MOISTURE FROM ABSOLUTE-HUMIDITY INDEX FOR CLEAR-CUT AREA

The regression equation given in the legend of figure 8 and the regression line shown in the figure, which are based on values computed

by standard methods from all available afternoon measurements for the clear-cut area, provide a means of estimating duff moisture content from air temperature and relative humidity combined in the form of the absolute-humidity index. The correlation coefficient for these two variables was found to be $+0.62$; since r^2 equals 0.38 , it is evident that only 38 percent of the variation in duff moisture is explained by the absolute-humidity index. Also, the standard error of estimate is ± 2.49 percent. Thus, large errors are necessarily associated with single estimates of duff moisture based on the absolute-humidity index. However, the standard error of an average of estimates varies in inverse proportion with the square root of the number of estimates averaged. Thus an average of 100 estimates of duff moisture has a standard error of ± 0.25 percent, and an average of 50 estimates has a standard error of ± 0.35 percent and hence is acceptable for use in rating the severity of a fire season.

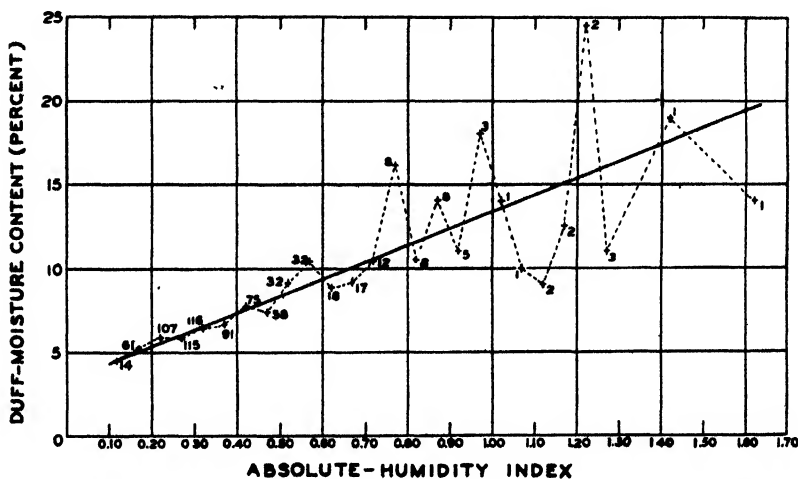


FIGURE 8.—Duff moisture and absolute-humidity index $\left(\frac{RH}{T}\right)$ on clear-cut area. (Basis, all available afternoon data, 793 observations taken at 4:30 p. m.) A = Relative humidity divided by temperature; X = duff moisture content; correlation coefficient $(r_{XA}) = +0.62$; standard error of estimate $(S_{r.A}) = \pm 2.49$ percent; regression equation is $X = 10.00A + 3.40$.

The low correlation and its high standard error of estimate preclude any possibility of substituting the simple measurement of temperature and relative humidity for the more difficult measurement of duff-moisture content in field practice. That is, duff moisture cannot be estimated from measurements of these two atmospheric factors with a degree of accuracy satisfactory for forest-protection purposes. Also, this method of estimating is entirely inapplicable on the day of a rain or on the day following a rain.

CORRELATIONS BETWEEN DUFF-MOISTURE CONTENT AND ABSOLUTE-HUMIDITY INDEX FOR HALF-CUT AND FULL-TIMBERED AREAS

Correlations between duff-moisture content and the absolute-humidity index are somewhat lower for the half-cut and full-timbered areas than for the clear-cut area. High fuel moistures tend to prevail on shaded areas. As is seen from figure 5, small changes in temperature and relative humidity have a greater effect on high fuel moistures

than on low ones. As a result, no doubt duff-moisture content is in equilibrium with the weather less often on timbered sites than in the open. Forest cover smooths out rapid fluctuations in weather much less than it retards the drying-out process in the fuels.

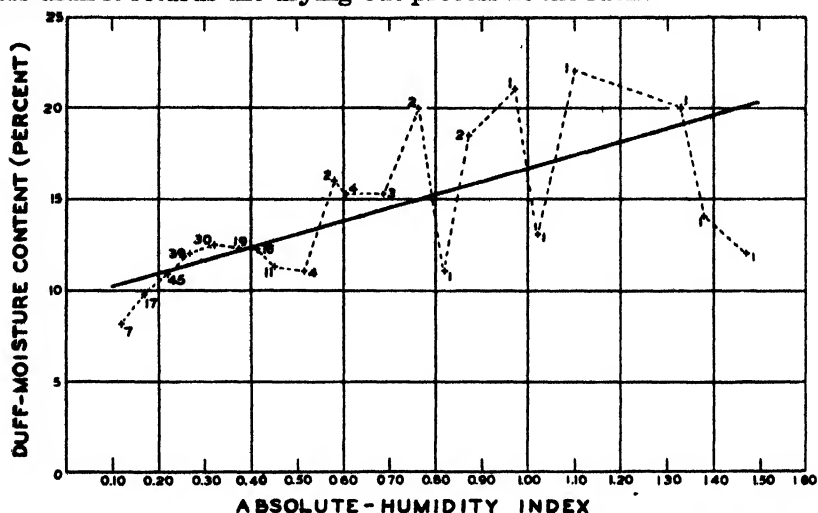


FIGURE 9.—Duff moisture and absolute-humidity index ($\frac{RH}{T}$) on half-cut area. (Basis, 206 observations taken at 4:30 p. m. not less than 5 days after 0.01 inch of precipitation.) A = Relative humidity divided by temperature; X = duff moisture content; correlation coefficient ($r_{x,A}$) = +0.47; standard error of estimate ($S_{x,A}$) = ± 2.78 ; regression equation is $X = 7.25A + 9.41$.

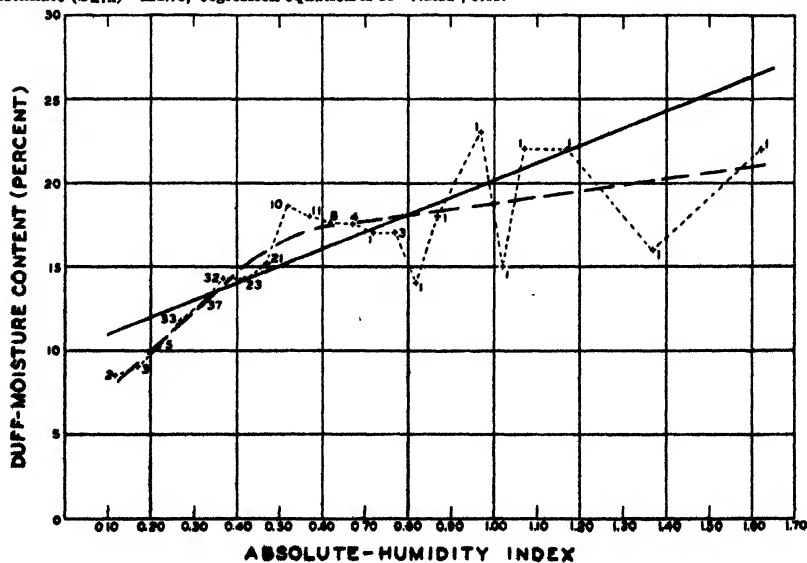


FIGURE 10.—Duff moisture and absolute-humidity index ($\frac{RH}{T}$) on full-timbered area. (Basis, 201 observations taken at 4:30 p. m., not less than 5 days after 0.01 inch of precipitation.) For linear regression, correlation coefficient ($r_{x,A}$) = +0.50; standard error of estimate ($S_{x,A}$) = ± 3.61 , and regression equation is $X = 10.23A + 9.90$; for curvilinear regression, correlation index ($\rho_{x,A}$) = 0.52, and standard error of estimate = ± 3.34 percent.)

Figures 9 and 10 show regressions, correlation coefficients, and standard errors of estimate computed from the afternoon data for

the half-cut and full-timbered sites after elimination of all observations made less than 5 days and less than 6 days, respectively, after 0.01 inch of precipitation.

On inspection of figures 9 and 10 a legitimate question arises, especially in the latter case, as to whether the curve of best fit is a straight line. A curve line was fitted to the data in figure 10 and its correlation index, symbolized by ρ , was found to be $+0.59$. A test of significance performed by Russell's method, previously described, revealed that there is no real difference between the correlation coefficient for the linear regression and the correlation index for the curve. Hence, the fit of the straight-line regression is satisfactory.

Correlations between duff moisture and the absolute-humidity index for the half-cut and full-timbered areas are of little practical value because of the high standard errors of estimate and because such correlations cannot be used until 5 days and 6 days, respectively, after 0.01 inch of precipitation.

CORRELATION OF DUFF MOISTURE WITH ABSOLUTE-HUMIDITY INDEX ON BASIS OF UNRESTRICTED DATA

The most usable method of estimating duff moisture from the absolute-humidity index would be one that could be applied on rainy days and on days immediately following rainy days, as well as at other times. By any method now available, little seems to be gained by making a correlation of duff moisture with the absolute-humidity index on the basis of data including measurements taken during and soon after rain. The effect of such procedure is to make the regression curvilinear. By the addition of free water to duff during rainy periods the duff-moisture content is increased much more than it could be increased by atmospheric humidity, even the increased humidity existing during such periods.

By inclusion of rainy-weather data in the basis of a correlation of duff moisture with absolute-humidity index for the clear-cut area, the correlation coefficient is increased from $+0.62$ to $+0.76$, but the standard error of estimate is increased from ± 2.49 percent to ± 9.19 percent. Thus an average of 100 observations based upon the curve would have the fairly large standard error of ± 0.92 percent.

CORRELATION OF YESTERDAY'S TEMPERATURE AND RELATIVE HUMIDITY WITH TODAY'S DUFF MOISTURE

The fact is well recognized that forest fuels do not respond instantly to sudden changes in the weather. The moisture content of any heavy fuel lags considerably. Since duff is an extremely light and porous material, its moisture content is more closely correlated with atmospheric conditions than that of other forest fuels; but even duff moisture is slower to change than humidity and temperature. A question arises as to whether duff moisture lags enough to justify correlating today's duff-moisture content with yesterday's weather.

The importance of yesterday's temperature and relative humidity as compared with today's in relation to current duff moisture was examined by analyzing the records made on the clear-cut area when the number of days since 0.01 inch of precipitation was 2 or more. In computing the absolute-humidity index different weights were given to the observations taken on the 2 days, respectively. For instance, in one correlation between duff moisture and absolute-humid-

ity index the latter was an average weighted in the ratio of 1 part for yesterday to 3 parts for today. This correlation produced a higher coefficient than any of the other combinations, $+0.71$; but the improvement in correlation indicated by this coefficient was not large enough to be significant. Hence, it appears that duff moisture on the clear-cut area lags behind the weather but slightly when the number of days since 0.01 inch of precipitation is 2 or more. This restriction on the data has, of course, excluded the influence of most periods of unsettled weather, in other words of most of those days when the greatest lag in duff moisture would be expected.

CORRELATION OF $\frac{1}{2}$ -INCH-WOOD MOISTURE AND ABSOLUTE-HUMIDITY INDEX

Two correlations were made between $\frac{1}{2}$ -inch-wood moisture and the absolute-humidity index by use of the afternoon measurements taken on the clear-cut area. In the first, all observations made less than 2 days after 0.01 inch of precipitation were rejected. The relation disclosed by this correlation (fig. 11), the regression for which

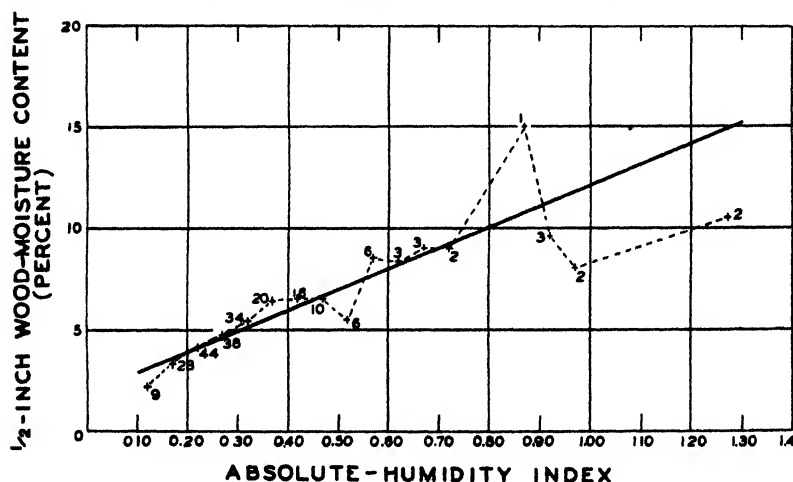


FIGURE 11.—Moisture content of $\frac{1}{2}$ -inch wood and absolute-humidity index ($\frac{RH}{T}$) on clear-cut area. (Basis, 222 observations taken at 4:30 p. m. 2 or more days after 0.01 inch of precipitation.) A = relative humidity divided by temperature. Y = $\frac{1}{2}$ -inch-wood moisture content. Correlation coefficient ($r_{Y,A}$) = $+0.68$. Standard error of estimate ($S_{Y,A}$) = ± 1.87 . Regression equation is $Y = 10.27A + 1.83$.

is linear, is slightly closer than that brought out by any of the correlations previously made; the correlation coefficient is $+0.68$, and the standard error of estimate is ± 1.87 percent. In the second correlation, use was made of all available afternoon data for the clear-cut area, including those that had been rejected in the first. As will be seen from figure 12, the regression is curvilinear. This is due almost entirely to the influence of precipitation.

An advantage and a disadvantage are associated with use of either of these two correlations for the purpose of estimating $\frac{1}{2}$ -inch-wood moisture from the absolute-humidity index. For estimates based on the curvilinear regression the coefficient was exceptionally high, $+0.90$, but the standard error of estimate was ± 7.46 . Thus whereas

an average of 100 estimates based on the linear regression would have a standard error of only ± 0.19 , an average of 100 estimates from the curve would have a standard error of ± 0.75 . The linear regression applies only when the number of days since 0.01 inch of precipitation is at least 2, but the curvilinear regression can be used for all days. There seems to be little choice between the two, since use of the first is restricted and use of the second permits reasonably accurate estimates only if at least 100 observations are averaged.

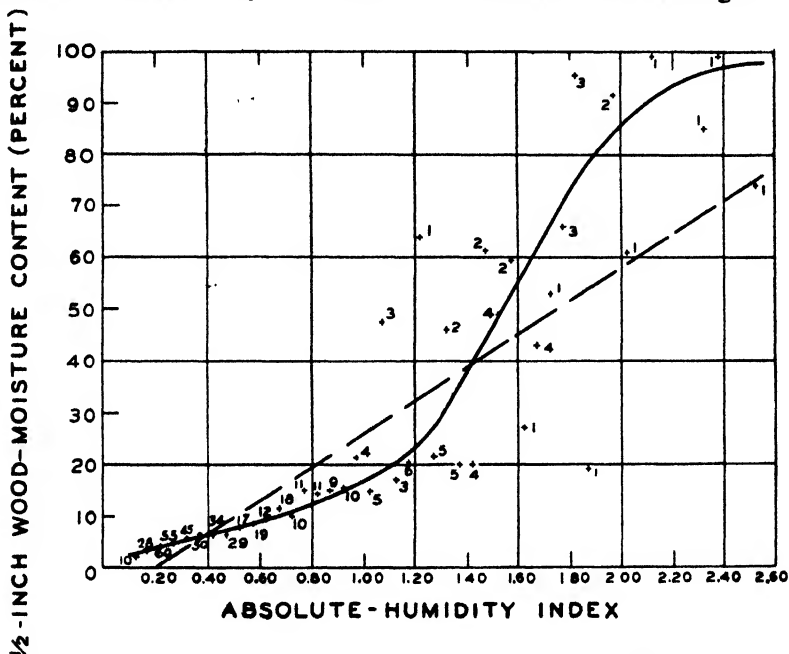


FIGURE 12.—Moisture content of $\frac{1}{2}$ -inch wood and absolute-humidity index $\left(\frac{RH}{T}\right)$ on clear-cut area. (Basis, all available afternoon data, 494 observations taken at 4:30 p. m.) For linear regression, correlation coefficient = +0.79, and standard error of estimate = ± 10.6 percent. For curvilinear regression, correlation coefficient = +0.90 and standard error of estimate = ± 7.46 percent.

RECOMMENDATIONS FOR FURTHER WORK

The results of this analysis suggest several recommendations for further work.

Although in this study correlation of total air movement during the daytime with the moisture content of lightweight fuels revealed no significant relation, it is possible that a significant relation exists between wind velocity during the hours of rapid drying only, say 9 a. m. to noon, and afternoon fuel moisture. The effect of wind should be further investigated.

It might be well, also, to investigate under northern Rocky Mountain conditions some variables not considered in this analysis, such as solar radiation and soil moisture. Results of a study by Stickel (10) indicated a slight correlation between these two factors and duff moisture in the Adirondacks. At present satisfactory methods of measuring these variables are wanting, especially in the case of soil moisture.

Some valuable information might be obtained through study of the correlation between fuel moisture and saturation deficit of the atmosphere; that is, the quantity of water vapor that would have to be added to the atmosphere at a given time in order to produce saturation. This factor provides a measure of the drying power of the atmosphere.

SUMMARY

In efforts toward satisfactory control of the forest fires that do tremendous damage in the United States each year, especially in the northern Rocky Mountain region, forest administrative officers are aided by current knowledge of fire danger—that is, of the sum total of the factors that determine whether fires will start, spread, and do damage. A complete understanding of current fire danger is difficult to obtain, because many factors are involved. By making observations or measurements of several of the most important factors and properly integrating them, however, the forest protectionist can determine the relative fire danger existing at a given time and place. Such a determination guides him in deciding whether and if so how much his force should be temporarily expanded or reduced, and how it should be distributed. In addition, through knowledge of the influence of given factors on fire danger the protectionist is assisted in interpreting weather forecasts and thus in preparing for all classes of fire danger that are likely to arise.

Fuel-moisture content has a very important bearing upon the ease with which fires start and the rate at which they spread. Weather, in turn, is the principal determinant of the moisture content of fuels. Current weather bears an especially close relation to the moisture content of duff and small branch wood, two fuels that are very widely distributed and that carry fire from tree to tree and from log to log. The relation between individual weather elements and the moisture content of these lightweight fuels must be understood before the proper weight can be assigned to each of the many weather factors that contribute to fire danger.

At the Priest River, Idaho, branch of the Northern Rocky Mountain Forest and Range Experiment Station, simultaneous daily measurements have been made, beginning in 1924, of duff-moisture content and the following weather elements and related factors: Maximum, minimum, and current air temperature; temperature of the dew point; current, minimum, and average relative humidity; precipitation, and the number of days since 0.01, 0.10, 0.20, 0.30, and 0.40 inch, respectively, of precipitation; and evaporation. Beginning in 1929, measurements were made also of $\frac{1}{2}$ -inch-wood moisture. Wind and maximum duff temperature were added in 1930. These measurements have been made on three adjacent sites in the western white pine type, approximately alike in all respects except that one is clear cut, one-half cut, and one fully timbered.

The fundamental relations between the individual weather elements measured and duff-moisture content for the clear-cut area were examined by means of multiple-correlation analysis. The results of the analysis do not apply to a rainy day or to a day immediately following rain. Data for such days were eliminated to avoid personal errors of fitting freehand curves and to facilitate analysis.

The analysis has definitely shown that of the weather elements and related factors examined current air temperature and relative humidity have the most important effect upon the moisture content of surface duff. Of 14 other weather factors studied, the most important were found to be wind, evaporation, duff temperature, temperature of the dew point, and number of days since 0.01 inch of precipitation. Current air temperature and relative humidity were found to explain all but about 3 percent of the total variance in duff-moisture content associated with those factors and the additional factors just listed. Thus inclusion of the latter in further correlations was impractical.

In this analysis great advantage resulted from the use of the variable $\frac{RH}{T}$, which represents the ratio of relative humidity to air temperature and constitutes an index of absolute humidity. One advantage foreseen in a method of estimating fire danger through use of this variable was that it would make possible fire-danger ratings for the past seasons during which records were made of air temperature and relative humidity but not of duff moisture.

Correlations of duff moisture with relative humidity and air temperature showed errors so large as to preclude all possibility that estimates of duff moisture based on simple measurement of humidity and temperature can be substituted satisfactorily in field practice for actual duff-moisture measurements. Averages for as many as 50 estimates based on these correlations, however, are acceptable for use in rating the severity of a fire season.

Coefficients of correlation of duff moisture with relative humidity and air temperature for the half-cut and full-timbered areas were slightly lower than those for the clear-cut area, and had larger errors associated with them. The usefulness of these correlations is restricted also by the fact that they do not apply until 5 days and 6 days, respectively, after rain.

Coefficients of correlation of $\frac{1}{2}$ -inch-wood moisture with relative humidity and air temperature for the three areas differed but little from those of duff moisture.

LITERATURE CITED

- (1) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.
- (2) GISBORNE, H. T.
1927. METEOROLOGICAL FACTORS IN THE QUARTZ CREEK FOREST FIRE. U. S. Monthly Weather Rev. 55: 56-60, illus.
- (3) ———
1928. MEASURING FOREST-FIRE DANGER IN NORTHERN IDAHO. U. S. Dept. Agr. Misc. Pub. 29, 64 pp., illus.
- (4) JEMISON, G. M.
1932. METEOROLOGICAL CONDITIONS AFFECTING THE FREEMAN LAKE (IDAHO) FIRE. U. S. Monthly Weather Rev. 60: 1-2.
- (5) ———
1934. THE SIGNIFICANCE OF THE EFFECT OF STAND DENSITY UPON THE WEATHER BENEATH THE CANOPY. Jour. Forestry 32: 446-451.
- (6) KINER, J. B., and MATTICE, W. A.
1928. STATISTICAL CORRELATIONS OF WEATHER INFLUENCE ON CROP YIELDS. U. S. Monthly Weather Rev. 56: 53-57, illus.
- (7) LARSEN, J. A., and DELAVAN, C. C.
1922. CLIMATE AND FOREST FIRES IN MONTANA AND NORTHERN IDAHO, 1909 TO 1919. U. S. Monthly Weather Rev. 50: 55-68, illus.

- (8) RUSSELL, E. W.
1933. THE SIGNIFICANCE OF CERTAIN "SINGLE VALUE" SOIL CONSTANTS.
Jour. Agr. Sci. [England] 23: [261]-310.
- (9) SHOW, S. B.
1919. CLIMATE AND FOREST FIRES IN NORTHERN CALIFORNIA. Jour.
Forestry 17: 965-979, illus.
- (10) STICKEL, P. W.
1931. THE MEASUREMENT AND INTERPRETATION OF FOREST FIRE-WEATHER
IN THE WESTERN ADIRONDACKS. N. Y. State Col. Forestry,
Syracuse Univ., Tech. Pub. 34, 115 pp., illus.
- (11) ———
1932. WEATHER AND FOREST FIRE HAZARD, WITH SPECIAL REFERENCE TO
THE WHITE PINE REGION OF CENTRAL NEW ENGLAND. Mass.
Forestry Assoc. Bull. 153, 8 pp., illus.
- (12) WALLACE, H. A., and SNEDECOR, G. W.
1925. CORRELATION AND MACHINE CALCULATION. Iowa Agr. Col. Off.
Pub. v. 23, no. 35, 47 pp., illus.

THE EFFECT OF DIRECTION OF ILLUMINATION UPON THE VISIBILITY OF A SMOKE COLUMN¹

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INTRODUCTION

Preliminary investigations in the detection of forest fires at the California Forest and Range Experiment Station were based on the assumption that the visibility of smoke columns in the field would vary as the visibility of the landscape with varying conditions of atmospheric suspensoid concentration and with the position of the sun. This led to the determination of the effect of direction of the sun with respect to the observer's line of sight upon the visibility of natural features in the landscape through analysis of a large number of lookout observations of natural targets on the Shasta National Forest. Later observations of test-fire smokes, however, indicated clearly that the visibility of smoke columns as reflected in fire-discovery time does not vary in the same manner as landscape visibility. The following laboratory experiment was devised, therefore, to determine in just what manner the visibility of a smoke column varies with different positions of the sun with respect to the observer's line of sight.

MATERIAL AND METHODS

With other factors constant, the visibility of a smoke column varies as a function of its brightness. In order to isolate and measure the variation in visibility due to changes in brightness, the variable influences of background, glare, and atmospheric suspensoid were eliminated from the experimental set-up.³ The two elements primarily concerned in the relationships to be determined, then, were a column of smoke and a light beam of parallel rays.

Figure 1 shows the arrangement in plan and elevation of the equipment as it was set up for the experiment. The beam of parallel rays was produced by a 400-watt projection lantern (a) with the projection lens removed, operating on an alternating-current circuit. A wooden frame in the form of a tank, 3 feet deep and 12 feet in diameter, was built and set 1 foot above the floor. This tank was

¹ Received for publication June 15, 1935; issued February 1936.

² The authors wish to acknowledge indebtedness to A. A. Brown, associate silviculturist at the California Forest and Range Experiment Station, who initiated the experiment, made arrangements for obtaining the laboratory in which it was performed, and gave considerable time to the general supervision of the work. They also wish to thank Professor Minor of the optometry division of the University of California for his interest and for suggestions offered in organizing the experiment, and Professor Boelter of the engineering department of the university for his cooperation and for his kindness in offering criticisms of the original manuscript.

³ Certain conclusions, however, relative to the effect of atmospheric suspensoid material upon visibility have been drawn from the laboratory observations.

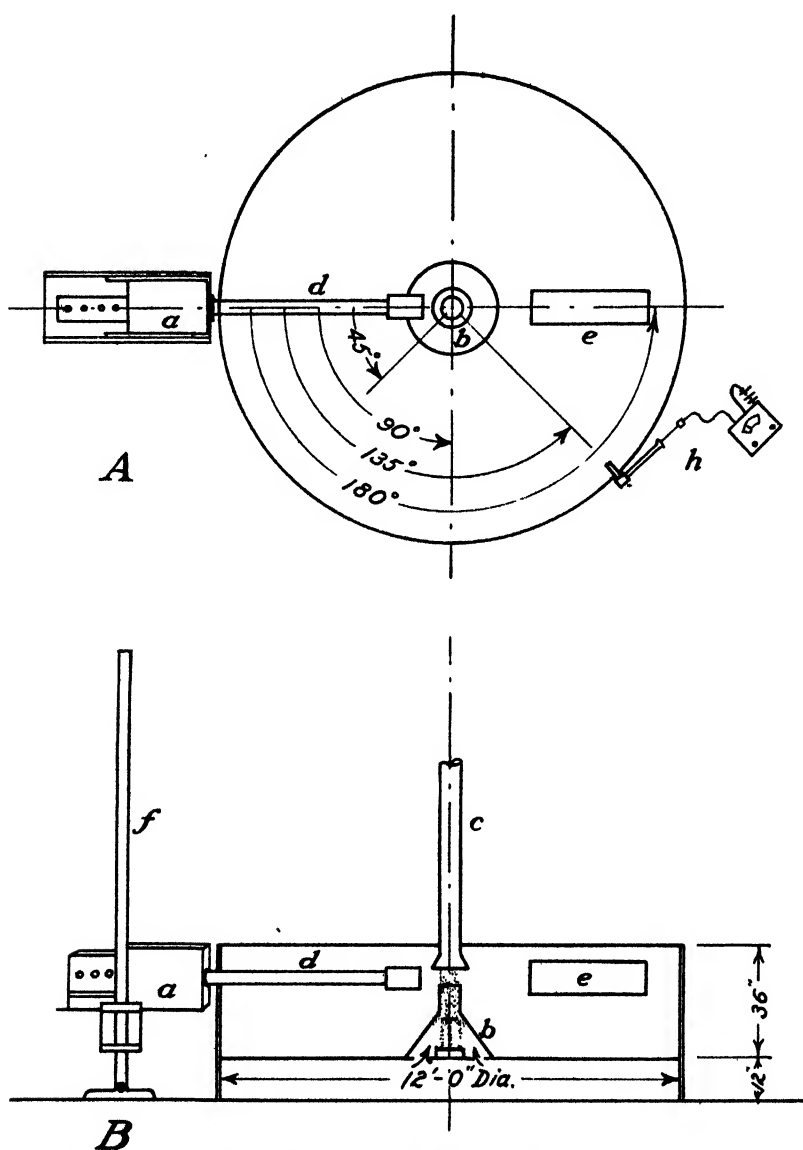


FIGURE 1.—Arrangement of experimental apparatus: A, Plan; B, elevation. Explanation in text.

lined with black builder's felt to eliminate variations in background and minimize the reflection of stray light. In the center of the tank was placed an inverted funnel-shaped hood (*b*) in which the smoke was produced. Eight inches above the top of the hood was placed a flue (*c*) to carry off the smoke and create a draft to keep the smoke in a uniform column. A specially constructed tube (*d*) was placed in front of the projector to adjust the size of the light beam and absorb the small amount of nonparallel rays produced by the projector. The beam of light was directed through the side of the tank at a height 2 inches above the top of the hood in the center of the tank. An 8-inch tube 3 feet long with one end closed (*e*) was hung in line with the beam of light to intercept it after it had passed through the smoke column, thus eliminating any reflection from the opposite side of the tank. The projector was mounted on an elevating frame (*f*) so that the light source could be adjusted to correspond with various elevations of the sun. All equipment was painted with flat black paint and observations were made in a totally darkened room.

The smoke column was produced by burning Chinese punk, the desired concentration of smoke being obtained by varying the number of punks burned at one time. A baffle plate at the lower end of the neck of the hood broke up the smoke and produced a uniform column $2\frac{1}{4}$ inches in diameter regardless of the number of punks burned. The punk burned at a uniform rate and produced a smoke similar in color to actual smokes observed in the field.

The diameter of the light beam was adjusted to the diameter of the smoke column so the apparent illuminated area of the smoke remained approximately the same regardless of the position from which it was observed.

A Macbeth illuminometer was used to measure the brightness of the illuminated smoke column. Measurements of smoke visibility given are based on the brightness of the smoke measured with this instrument in international candles per square foot.

Measurements of brightness and visibility of the illuminated smoke column were made at observation stations placed at intervals of $22\frac{1}{2}^{\circ}$ around the tank in the same horizontal plane as the illuminated portion of the smoke column. These stations are designated by the angle *abh* in the diagram, in which the illuminometer (*h*) is shown at station 135° .

EXPERIMENTAL DATA

In the preliminary laboratory tests, observations with the Bennett-Casella visibility meter,⁴ used for visibility measurements in the field, were made simultaneously with observations with the Macbeth illuminometer in order to establish the relationship between the brightness of the smoke and the visibility-meter readings with a black background. Table 1 is a summary of the average brightness

⁴ BENNETT, M. G. A VISIBILITY METER. Jour. Sci. Instruments 8 (4): 122-126. 1931.

values obtained. In figure 2 visibility-meter readings are plotted over these values on a logarithmic scale. Since the plotted points fall along a straight line it follows that the visibility-meter readings are a simple multiple of the logarithm of the brightness.

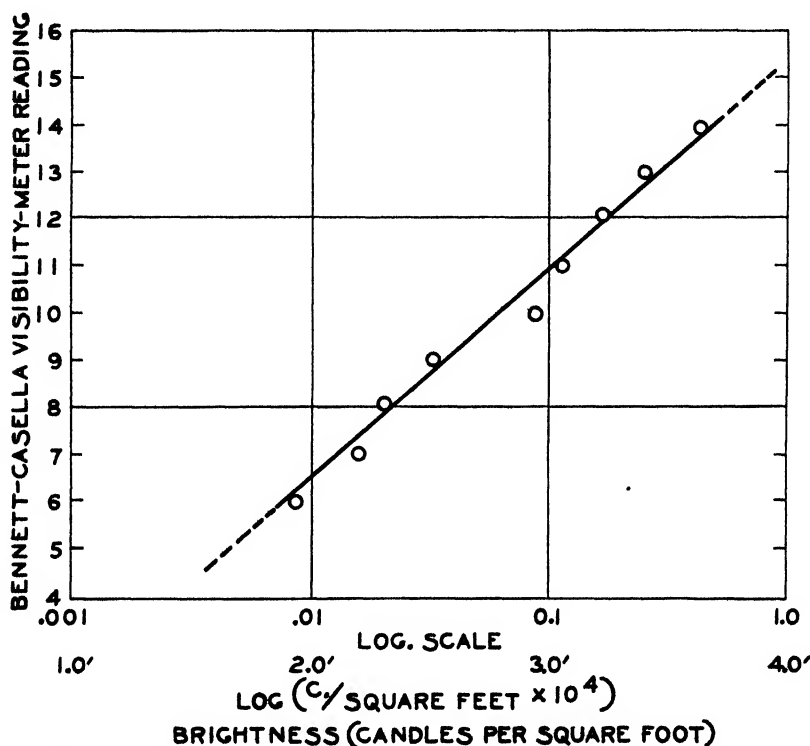


FIGURE 2.—Relationship of Bennett-Casella visibility-meter reading to brightness of smoke column.

TABLE 1.—Relationship between Bennett-Casella visibility-meter readings and brightness measurements of smoke columns

Visibility-meter reading	Brightness (candles per square foot)	Log ($\frac{\text{candles}}{\text{square foot} \times 10^4}$)
6.....	0.0087	1.940
7.....	.0158	2.199
8.....	.0198	2.297
9.....	.0314	2.497
10.....	.0825	2.916
11.....	.1140	3.057
12.....	.1680	3.225
13.....	.2490	3.396
14.....	.4210	3.624

There is a decided inequality in the graduations of opacity of the various filters in the visibility meter, resulting in the failure of the plotted values in figure 2 to fall exactly on a straight line. This inequality in the steps of opacity also gives visibility-meter readings

slightly too low for low values and approximately the same amount too high for high values (fig. 3). The approximate relationship indicated, however, when brightness is expressed in candles per square foot, is—

$$V = 3.6 \left[\log \frac{(\text{candles})}{(\text{square foot})} \times 10^4 \right]$$

where V is visibility-meter reading.

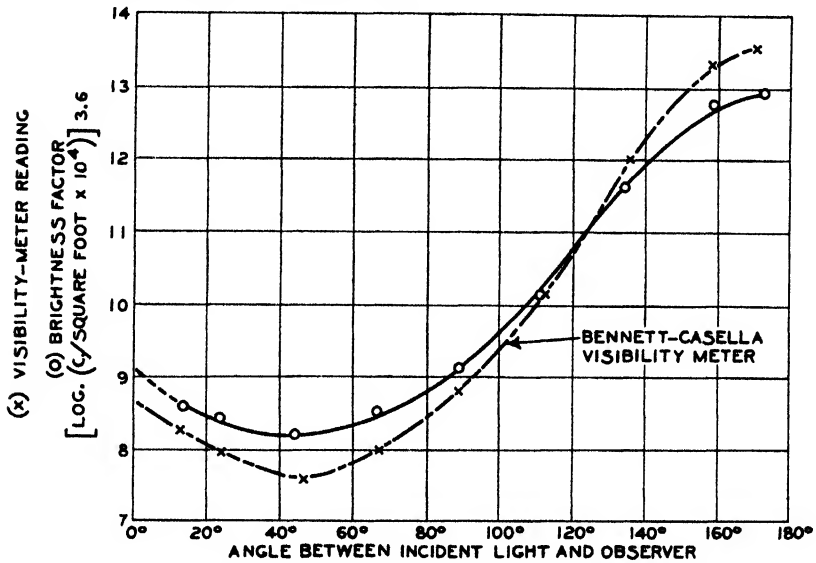


FIGURE 3.—Effect of direction of illumination on the visibility of a smoke column.

In the preliminary observations a large number of brightness measurements was made with the line of sight in a horizontal plane and the light source in positions varying from horizontal to a vertical

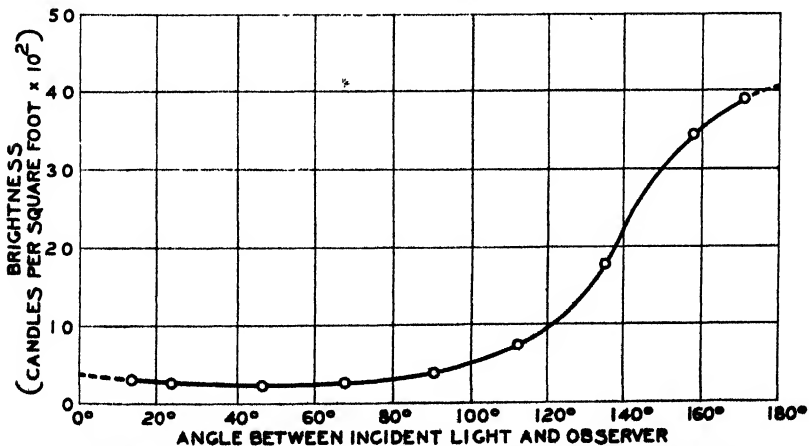


FIGURE 4.—Effect of direction of illumination on the brightness of a smoke column.

angle of 50° with respect to the smoke column, and the effect of direction of illumination on the brightness of the smoke column was

determined. Considerable variation was noted in brightness measurements taken at different times, resulting in differences in the numerical values obtained. A representative set of measurements is given in table 2 and shown graphically in figures 3 and 4. The angles indicated are the angles measured at the smoke between the light source and the observer. Visibility-meter readings given are determined from the brightness measurements through the relationship shown in figure 2. This was done to smooth out the roughnesses in individual visibility-meter readings.

TABLE 2.—*Effect of direction of illumination upon the brightness of a smoke column*¹

Station (degrees)	Illuminometer reading ²	Brightness candles (square foot)	Log (B×10 ⁴)	[Log (B×10 ⁴)]×3.6	Visibility-meter reading
0.0.....		0.0300	2.477	8.9	8.6
11.25.....	3.10-49	.0249	2.396	8.6	8.2
22.5.....	2.76-49	.0216	2.334	8.4	8.0
45.0.....	2.24-49	.0176	2.246	8.1	7.6
67.5.....	2.81-49	.0220	2.342	8.4	8.0
90.0.....	4.23-49	.0331	2.520	9.1	8.8
112.5.....	8.71-49	.0684	2.835	10.2	10.
135.0.....	6.16-12	.1710	3.233	11.6	12.
157.5.....	12.30-12	.3410	3.533	12.7	13.
170.0.....	14.05-12	.3890	3.590	12.9	13.
180.0.....		.4000	3.602	13.0	13.

¹ Smoke density = 5 punks; vertical angle: incident light = 0°, observation = 0°. Variation in illumination at smoke column = 91.6 to 90.0 foot-candles. Tabulated values in column 2 are averages of values taken at plus and minus horizontal angles; values for 0° and 180° in column 3 are extrapolated; values in column 6 taken from figure 2.

² Filter no. 49—candles/square foot = reading × 0.00784; filter no. 12—candles/square foot = reading × 0.0277

Several sets of observations resulted in variations in the numerical values of brightness obtained, but the trends of increasing brightness with increasing angle were all of the same shape. These variations were traced to two varying factors in the set-up: Light intensity of the source, affected by changing voltage in the light circuit; and density of the smoke column, affected by changes in the draft up the flue, which depended both upon outside air temperature and temperature of the room. To eliminate these variations, a voltmeter was placed in the light circuit and a photronic cell³ in the light beam behind the smoke column. It was assumed that when the voltage remained constant any variation in the reading of the photronic cell was caused by variation in the smoke density. In the final observations readings were taken only when the voltmeter and photronic cell measured constant values.

After the trend of brightness had been established for various angles between the light source and the observer with both in a horizontal plane, several series of observations were made with the line of sight of the observer in a horizontal plane but with the light source raised to various vertical angles to correspond with the varying elevations of the sun. The results obtained from these observations did not check with computed values, for the reason that elevating the light source increased the volume of smoke illuminated, thus increasing its apparent brightness. In the field, where the entire smoke column is in sunlight, an increase in the elevation of the sun does not increase the volume of smoke illuminated. With the light source in a horizontal plane, a series of measurements of brightness was made from

³ GOODWIN, W. N., JR. THE PHOTRONIC ILLUMINATION METER. Illuminating Engin. Soc. Trans. 27: 823-835, illus. 1932.

various vertical angles at different horizontal stations. The values thus obtained corresponded with the values obtained in a horizontal plane at an angle equal to the "spherical" angle between the light source and the observer. These observations indicated that the angle measured at the smoke column between the light source and the observer determines the effect of direction of illumination on the visibility of a smoke column regardless of the azimuths and elevations of any of the three points. All later observations were taken in a horizontal plane, since the horizontal angles between light source and observer could be substituted for "spherical" angles directly. In figure 5 is shown the effect of direction of illumination for different

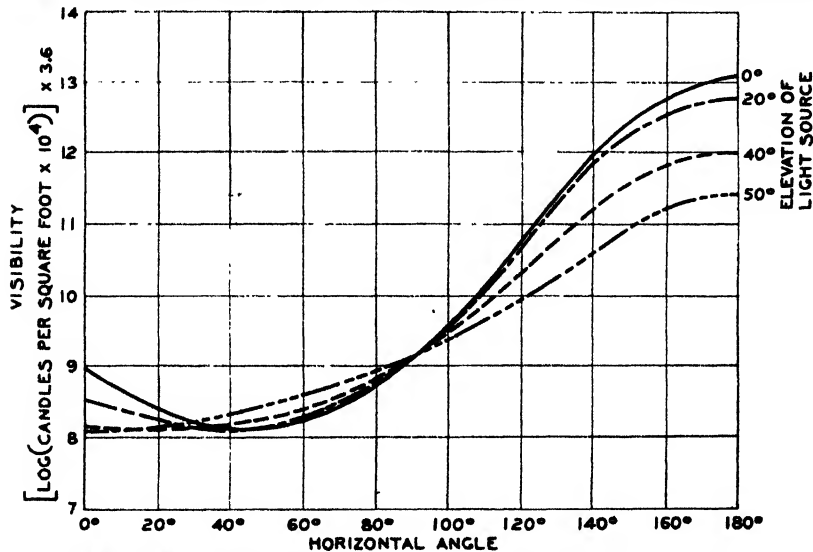


FIGURE 5.—Effect of different elevations of the light source on visibility of a smoke column.

elevations of the light source. Table 3 gives the equivalent angles for different horizontal and vertical components.

TABLE 3.—Angle between light source and observer for given horizontal and vertical angle components

Horizontal angle (degrees)	Equivalent for vertical angle (degrees) indicated									
	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°
0.....	5	10	15	20	25	30	35	40	45	50
10.....	12	15	19	23	27	32	37	42	47	52
20.....	21	23	26	29	32	36	40	45	49	54
30.....	31	32	34	36	39	42	46	50	54	57
40.....	41	42	43	45	47	50	52	55	57	61
50.....	51	52	53	54	55	57	59	61	63	66
60.....	60	61	61	62	63	65	66	68	70	72
70.....	70	71	71	72	72	73	74	75	76	78
80.....	80	80	80	81	81	82	82	83	83	84
90.....	90	90	90	90	90	90	90	90	90	90
100.....	100	100	100	99	99	99	99	98	98	97
110.....	110	110	110	109	109	108	108	106	105	104
120.....	120	120	120	119	118	116	115	113	112	110
130.....	130	130	129	128	126	124	122	120	118	116
140.....	140	140	139	137	135	132	130	127	124	122
150.....	150	150	148	145	143	140	137	134	130	125
160.....	160	160	157	153	150	146	142	138	134	128
170.....	170	170	167	162	158	150	146	140	136	130
180.....	175	170	165	160	155	150	145	140	135	130

Measurements of the effect of concentration of the smoke column on its brightness and on its light-transmission factor were made at

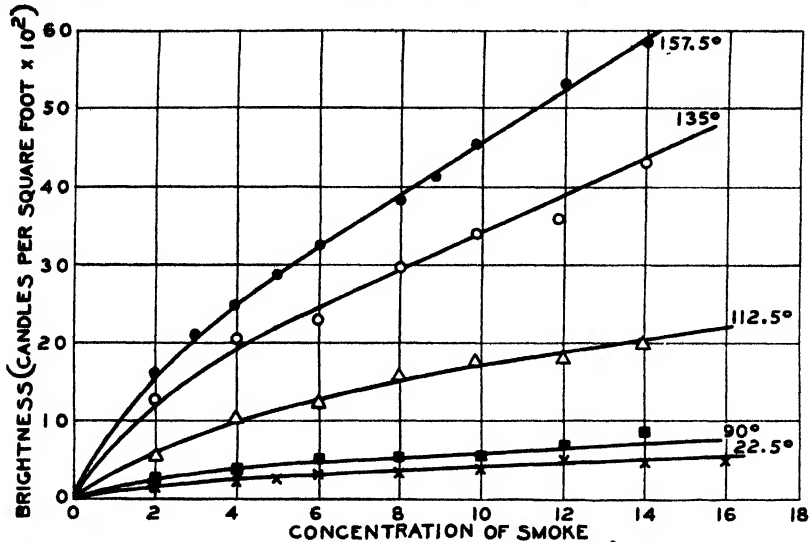


FIGURE 6.—Effect of concentration of smoke on its brightness for different angles (indicated on curves) between (horizontal) light source and observer.

the same time. The concentration of smoke was varied by placing additional punks under the hood, the number ranging from 1 to 14. The heat caused by more than this number produced an exceptionally

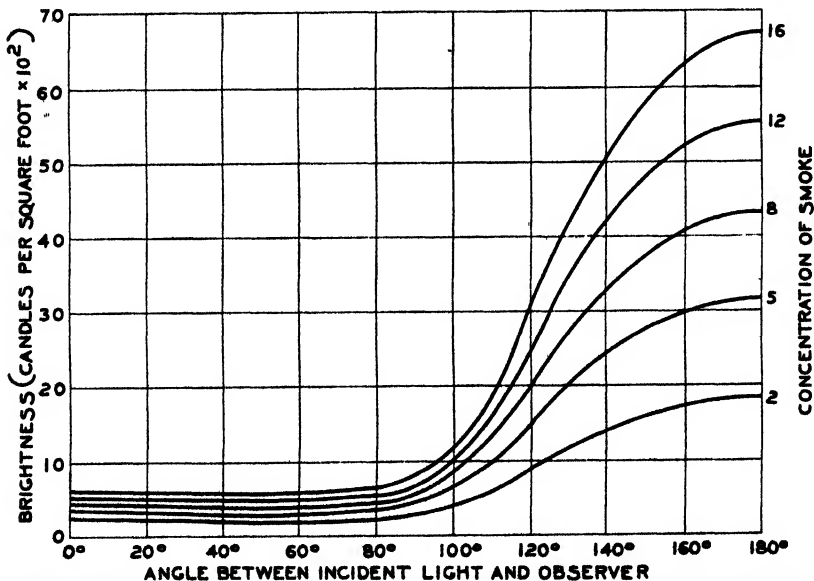


FIGURE 7.—Effect of direction of illumination on brightness of a smoke column for different concentrations of the smoke.

strong draft, which resulted in discrepancies in both brightness and light-transmission measurements. Readings of brightness were taken

at five stations for each change in concentration of the smoke (table 4). These values are shown graphically in figure 6. Figure 7 is derived from figure 6 by cross-plotting the five curves in the latter.

The photronic cell was used to record the foot-candles of light passing through the smoke column. For each change in the concentration of the smoke, as determined by the number of punks burned, the meter of the photronic cell was watched, and the reading that seemed to represent the most stable and uniform condition of the smoke was tabulated. These values for the various concentrations are given in table 5 with their corresponding percentages of light transmission, which are presented graphically in figure 8.

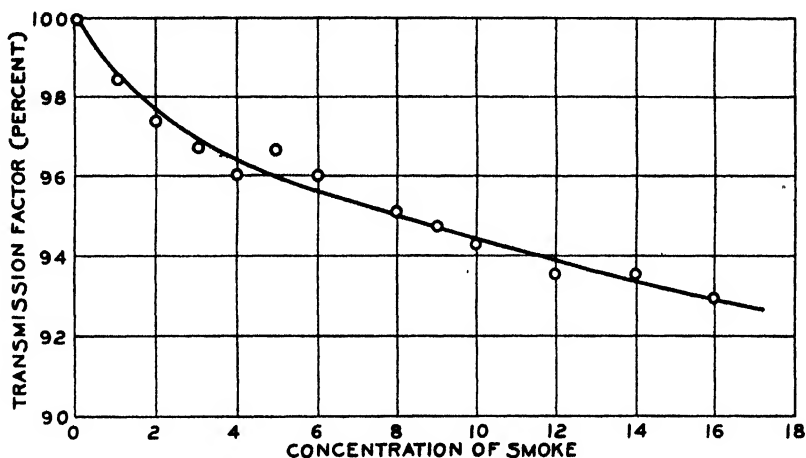


FIGURE 8. — Effect of concentration of a smoke column on its transmission of light.

TABLE 4.—*Effect of concentration of the smoke on the brightness of a smoke column at different angles between light source and observer*¹

Concentration of smoke column (number of punks)	22.5°		90°		112.5°		135°		157.5°	
	Illumi- nometer reading	Bright- ness ²	Illumi- nometer reading	Bright- ness ²	Illumi- nometer reading	Bright- ness ²	Illumi- nometer reading	Bright- ness ²	Illumi- nometer reading	Bright- ness ²
2.....	1.9-49	0.0149	3.3-49	0.0259	2.1-12	0.0582	4.6-12	0.128	6.0-12	0.166
3.....	3.3-	.0259	4.0-	.0314				7.75-		.215
4.....	3.0-	.0236	4.7-	.0368	4.0-	.1110	7.75-	.215	9.0-	.250
5.....	3.55-	.0279	5.3-	.0416				10.6-		.293
6.....	3.6-	.0282	5.37-	.0421	4.6-	.1280	8.3-	.230	11.75-	.325
8.....	4.4-	.0345	5.8-	.0455	6.1-	.1690	10.6-	.294	13.4-	.372
9.....	5.6-	.0439	6.75-	.0530					15.0-	.416
10.....	4.4-	.0345	6.8-	.0532	6.1-	.1690	12.4-	.344	16.5-	.457
12.....	6.9-	.0541	9.0-	.0706	6.5-	.1800	12.6-	.349	19.2-	.532
14.....	6.2-	.0496	11.5-	.0902	7.1-	.1970	15.4-	.427	6.5-49	.586
16.....					7.3-	.2020				
18.....							18.0-	.498		

¹ Illumination at smoke column: 130.0 foot-candles.

² Candles
Square foot

TABLE 5.—*Effect of concentration of a smoke column on transmission of light*

Concentration of smoke column (number of punks)	Light transmitted	Transmission factor	Concentration of smoke column (number of punks)	Light transmitted	Transmission factor	Concentration of smoke column (number of punks)	Light transmitted	Transmission factor
	<i>Foot-candles</i>	<i>Percent</i>		<i>Foot-candles</i>	<i>Percent</i>		<i>Foot-candles</i>	<i>Percent</i>
0.....	33.1	100.0	5.....	32.0	96.7	10.....	31.2	94.2
1.....	32.6	98.5	6.....	31.8	96.0	11.....		
2.....	32.2	97.4	7.....			12.....	31.0	93.6
3.....	32.0	96.7	8.....	31.5	95.1	13.....		
4.....	31.8	96.0	9.....	31.4	94.8	14.....	31.0	93.6

No measurements were made of the effect of suspensoid in the atmosphere, since there was no way in which the suspensoid could be illuminated uniformly in the laboratory. The foot-candles of light from a point light source decrease as the square of the distance from the source; hence the atmospheric suspensoid would be much more strongly illuminated near the light source than at some distance from it. This relationship was checked in the laboratory. For the same reason no observations were made with illuminated backgrounds.

It was observed that the veiling brightness of the illuminated atmospheric suspensoid increased in the same manner as the brightness of the smoke column when the angle of observation with respect to the light source was increased. Incidentally it was noted that a smoke column illuminated only by diffused light was visible through a considerable density of atmospheric suspensoid as long as the line of sight through the suspensoid was in shadow. When the line of sight passed through a short distance of illuminated suspensoid, however, the smoke column became decidedly less visible even though the observer was in shadow. The observer here remained in shadow to eliminate the effect of glare.

DISCUSSION

Before any general conclusions can be drawn, some explanation of the various measurements of brightness and visibility must be made. In the laboratory, color contrasts were not considered and background was held constant with a brightness of zero. The visibility of the illuminated smoke column was therefore a function of its brightness.

According to Fechner's law, the eye reacts to variations in the stimulus as the logarithm of the stimulus. Thus the visibility of the smoke column varies as the logarithm of the brightness. A comparison of figures 3 and 4 indicates the relationship between the brightness of the illuminated smoke column and its visibility. The brightness measured in absolute units in figure 4 increases, from a minimum at an angle between light source and observer of 45°, to approximately 19 times this value at an angle of 170°. In figure 3, however, when the logarithmic values are plotted, it is seen that the visibility increases only by approximately 62½ percent in the same interval. A further study of figure 4 indicates that Bennett-Casella visibility-meter readings are approximately in proportion to the visibility of the smoke column.

Some consideration should be given to the cause of the phenomenon of increasing brightness with increasing angle between the light source and line of sight of the observer, as illustrated in the curves of figure 7. The brightness of the smoke column observed at angles of less than 90° is caused principally by light reflected by the smoke particles, while at angles greater than 90° the brightness is caused largely by transmitted scattered light. Since the smoke column transmits much more light than it reflects, the brightness of the column is considerably greater when observed from the larger angles. Light incident on a smoke column is either reflected or scattered and at least partially polarized, depending on the size and character of the individual smoke particles; and the light reflected or scattered by each particle may be reflected or further scattered many times by additional particles in its path. The minimum brightness measurements obtained were recorded at station 45° , the sum of the multiple reflected and scattered light being at a minimum at this point. This causes the curves to take the form of a trigonometric function of the angle between light source and observer 45° out of phase.

The phenomenon of increasing brightness of smoke with increasing angle between the sun and observer is of importance because it not only determines the relative intrinsic visibility of a column of smoke as determined by the direction from which the smoke is observed, but has a strong influence on the detrimental effect on visibility of haze in the form of solid suspensoid material in the atmosphere.

In addition to producing a veiling brightness which lowers visibility by decreasing the contrast between the smoke column and its background, suspensoid material in the atmosphere also decreases the visibility of an object by decreasing the transmission of light reflected from the object through the atmosphere to the observer. Figure 8 illustrates the manner in which this transmitted light is decreased by various concentrations of suspensoid material in a smoke column. It is assumed here that light transmission through a longer distance of less concentrated suspensoid would follow the same trend as that indicated for the smoke column. Further laboratory work is now being planned to determine the relative effects of concentration and distance upon the transmission of light through an atmosphere containing suspensoid material. The relatively high light-transmission factor of a smoke column obtained in the experiment indicates that in general the detrimental effect on visibility of an atmospheric suspensoid is probably more attributable to veiling brightness than to decreased light transmission.

CONCLUSIONS

Although it is unlikely that relationships observed in the laboratory would ever be as sharply defined in the field, where a large number of variable factors is inevitably encountered, the same trends will be followed. The results of a series of laboratory experiments to determine the effect of certain factors on the visibility of a smoke column may be given, then, in terms of field observations, as follows:

- (1) The angle measured at the smoke between the sun and the observer determines the effect of direction of illumination on the visibility of a smoke column regardless of its azimuth or position of the sun as determined by the time of day.

(2) In an atmosphere containing little or no suspensoid material, the visibility of a smoke column is influenced appreciably by the relative position of the sun with respect to the line of sight of an observer. There is little variation in the visibility of the smoke column with changes in the angle between the incident light and line of observation from 0° to 90° —that is, with the sun varying in position from directly behind the observer to a line at right angles with his line of sight. When the angle is greater than 90° there is a very marked progressive increase in the visibility of the smoke until the observer is looking almost directly into the sun. The smoke is not visible when the sun forms its background.

(3) When there is an appreciable amount of suspensoid material in the atmosphere there is a decrease in the visibility of a smoke column viewed through the atmosphere owing to a combination of two factors—decreased light transmission factor of the atmosphere and veiling brightness of the suspensoid material. The first factor is constant for a given suspensoid concentration, while the second is present only when the suspensoid is illuminated and varies not only with suspensoid concentration but also with direction of illumination with respect to the observer's line of sight.

(4) The visibility of an object is decreased when the concentration of atmospheric suspensoid through which the object is viewed is increased. When the sun is behind or at right angles to the observer's line of sight, this decrease in visibility may be caused largely by decreased light transmission of the atmosphere, but with angles between the sun and observer greater than 90° , the detrimental effect of veiling brightness on visibility is much greater.

(5) The increasing brightness of suspensoid particles with increasing angle between the sun and observer's line of sight definitely affects the ease with which fires may be detected by lookout observers. When there is present an appreciable concentration of atmospheric suspensoid material, the visibility of landscape features in the general direction of the sun is decreased by the veiling brightness of the suspensoid material. The same factor, however, which causes the increased brightness of the atmospheric suspensoid also increases the brightness of a smoke column observed in that direction. The net result, then, is that even with a relatively high concentration of atmospheric suspensoid material present, smoke columns may be detected more readily and at greater distances in the general direction of the sun than in the opposite direction.

TOXICITY OF ALUMINUM SALTS TO TOBACCO PLANTS¹

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INTRODUCTION

It has been suggested that certain diseases which affect tobacco when it is grown after corn, timothy, or red clover may have their origin in the soluble aluminum salts of the soil. The principal difficulty in such rotations has been the development of the so-called "brown root rot" of tobacco. The present study was undertaken to determine whether soluble aluminum salts have the capacity to engender this disease.

The relatively large amount of aluminum in the earth's crust suggests that a considerable amount of this element would be found in the soil solution. However, the extreme insolubility of aluminum silicates prevents a rapid accumulation of aluminum in the ionic state. This means that aluminum salts of the weak acids are not highly soluble. Thus it may be anticipated that conditions for soluble aluminum would be more favorable when the hydrogen-ion concentration of the soil is high and the concentration of other salts usually found in soil is low.

EFFECT OF ALUMINUM ON TOBACCO PLANTS GROWN IN SOLUTIONS

Tobacco planted on September 18, 1934, and harvested on December 6, 1934, was grown in a complete nutrient solution (Knop's solution) on alternate 2 days, and during the intervening 2 days in various solutions of aluminum citrate. The containers were of 1-quart capacity. During the entire period Knop's solution with a trace of boric acid was used as the standard and in this the controls were grown. Five plants were grown in each solution. The quantity of aluminum taken up by the roots of the plants in the different solutions was as follows:

Percentage proportions of 0.006 molar aluminum citrate, added to culture solutions	Parts per million of aluminum in roots of tobacco plants	Percentage proportions of 0.006 molar aluminum citrate, added to culture solutions	Parts per million of aluminum in roots of tobacco plants
Control.....	3	50.....	503
0.1.....	1	70.....	569
2.....	6	85.....	587
5.....	12	95.....	893
15.....	361	98.....	2,165
30.....	502	100.....	2,165

¹ Zero indicates distilled water.

Plants grown in aluminum citrate showed definite signs of toxicity. The general toxic effect was more pronounced in the roots; but, as

¹ Received for publication Apr. 23, 1935; issued February 1936. Published as contribution no. 211 of the Massachusetts Agricultural Experiment Station.

compared to the controls, the tops showed a slightly more retarded growth than the roots. The most marked retarding influence of aluminum citrate began to be apparent in concentrations between 0.0009 M and 0.0018 M. Among the plants in other experiments, including plants in the seedling stage, the decrease in growth rate was more rapid at the above-mentioned concentrations. Thus the retardation in growth rate was greater with the percentages between 15 and 30 percent of the total concentration 0.006 M; that is, approximately 24 to 48 parts per million of aluminum. At concentrations higher than this the plants showed only moderately increased effects of toxicity up to 162 parts per million, or 100 percent of 0.006 M. McLean and Gilbert² found that aluminum in aluminum citrate, in quantities from 3 to 13 parts per million, was stimulating to plant life, while in larger amounts it was toxic.



FIGURE 1.—Tobacco plants grown for alternating 2-day periods in complete nutrient solutions and in water containing various percentage proportions of 0.006-molar aluminum citrate solution: No. 1, control; no. 2, 0 (distilled water); no. 3, 2; no. 4, 5; no. 5, 15; no. 6, 30; no. 7, 50; no. 8, 70; no. 9, 85; no. 10, 95; no. 11, 98; no. 12, 100 percent of 0.006-molar aluminum citrate solution

The plants grown in distilled water in alternate 2-day periods and in a complete nutrient solution in the intervening 2-day periods, showed retarded growth rate as compared with the plants grown in a complete nutrient solution during all the days of experimentation. The plants which received aluminum in relatively small amounts, as 0.00012 M, showed no stimulation of growth because of the presence of aluminum.

The plants affected by aluminum acquired a darker foliage than normal plants and the tops were not as large (fig. 1). These tops did not appear to be diseased or abnormal like those of plants influenced by single ions of the alkalis, or alkali earths, but appeared to be suffering from a lack of nutrients. The toxicity of the aluminum to the root was manifested by the retardation of growth of the primary root, and in cases of more definite and severe toxicity the lesion became so intensified that it assumed the nature of an abscission with tissue sloughing off and lateral roots taking the place of the primary one. In cases of more marked injury of root tip, when the solution was changed from aluminum only to complete nutrient solution, a white flocculent substance formed on the end of the injured root. This was apparently in part a phosphate or hydrosol of aluminum formed from the drop adhering to the injured root from the previous aluminum

² McLEAN, F. T., and GILBERT, B. E. ALUMINUM TOXICITY. *Plant Physiol.* 3: 293-302. 1928.

solution and in part an exudate from the injured tissue. The aluminum is apparently located in the superficial layers of the root and eventually leads to reduced intake of nutrients.

Although local brown areas were observed, there was no general browning of the roots as is the case when brown root rot occurs in fields. In other words, there appeared to be no relation between the toxic effect of aluminum and the symptoms of brown root rot.

The lethal dose of aluminum citrate for tobacco is not known. The plants when removed from the toxic solution and placed in a more favorable medium seem to have marked capacity for recovering. Lateral roots are thrown out above the water line of the nutrient solution, and only the portion in the toxic solution seems to be affected.

It would seem that the presence of excessively large amounts of aluminum in plants grown in solutions of relatively high concentrations of aluminum citrate is due in part to imbibition only to areas characterized as superficial layers of tissue.

It is apparent from the work of previous investigators, and from the present investigation, that aluminum occurs in greater abundance in the roots than in the tops. With this in mind an analysis was made of aluminum in the roots of tobacco plants grown at the different aluminum concentrations shown on page 519. The method used was that of Winter, Thrun, and Bird,³ according to which the colors developed in the presence of aurintricarboxylic acid are compared. It was found that much aluminum was taken up by the plants growing at high concentrations of aluminum (fig. 1). The high values of aluminum in the plants began when they were grown at 15 percent of the 0.006 molar concentration, and this is the percentage at which the growth curve begins to fall perceptibly. At lower concentrations there was no great toxicity. At 15-percent concentration there was present in the solution an approximate total of 24 parts per million of aluminum. At the extreme high concentration there was a large intake of aluminum without a subsequent pronounced lowering of the growth curve. The tops of the plants contained less aluminum than the roots, but the amounts did not seem to be in proportion to the quantity added to the solution. The small amount of aluminum found in the plants grown in distilled water and in the controls may probably be accounted for by the fact that these plants had grown in soil before they were transplanted, and had taken up aluminum from the soil at a very early stage of growth.

EFFECT OF CALCIUM IN CORRECTING THE TOXICITY OF ALUMINUM TO TOBACCO PLANTS GROWN IN SOLUTIONS

It has been assumed that ionic calcium has the capacity to counteract the unfavorable effects induced by an unbalanced proportion of other ions. To determine the validity of this assumption a series of tests was made. Calcium nitrate and aluminum citrate were used in varying proportions in a complete nutrient solution. Aluminum citrate does not readily precipitate over a wide range of hydrogen-ion concentrations. There were eight sets of molecular proportions of the two salts calcium nitrate and aluminum citrate as shown in table 1, which gives the percentage proportions of 0.006 M of each salt.

³ WINTER, O. B., THRUN, W. E., and BIRD, O. D. THE DETERMINATION OF ALUMINUM IN PLANTS. I. A STUDY OF THE USE OF AURINTRICARBOXYLIC ACID FOR THE COLORIMETRIC DETERMINATION OF ALUMINUM. *Jour. Amer. Chem. Soc.* 51: 2721-2731, illus. 1929.

The test plants were grown in solutions which were like those of the controls except that they contained varying amounts of aluminum and calcium, and four times as much potassium nitrate. Thus, 0 to 100 percent of aluminum citrate and an inverse percentage proportion of calcium nitrate. The plants used as controls were grown in Knop's solution without modification. The solutions were changed twice a week. The age of plants when harvested was 2½ months. Five plants were used for each concentration.

In those solutions containing the maximum percentage proportions of aluminum citrate the reaction was approximately equivalent to a pH of 4, while those with minimum amounts of the salt had a pH equivalent to about 5.9.



FIGURE 2.—Tobacco plants grown in medium in which only calcium nitrate and aluminum citrate were varied, the other nutrients being kept constant. The following numbers express the relative percentage proportions of 0.006 molar calcium nitrate and of 0.006 molar aluminum citrate used: 1, Control; 2, 100 percent calcium nitrate+0 percent aluminum citrate; 3, 95 percent calcium nitrate+5 percent aluminum citrate; 4, 85 percent calcium nitrate+15 percent aluminum citrate; 5, 50 percent calcium nitrate+50 percent aluminum citrate; 6, 25 percent calcium nitrate+75 percent aluminum citrate; 7, 15 percent calcium nitrate+85 percent aluminum citrate; 8, 5 percent calcium nitrate+95 percent aluminum citrate; 9, 0 percent calcium nitrate+100 percent aluminum citrate.

The plants receiving no calcium were stunted, as indicated by their roots and tops. Where a small amount of calcium was added (0.0003 M $\text{Ca}(\text{NO}_3)_2$), the tops were approximately 3 times as tall as those of plants in solutions containing no calcium, and the dry weight of these plants was 9 times that of those in the calcium-free solution.

The most perceptible effect of low calcium on the tops was the retarded development of the primordial meristem. The leaves at the apex of the plant seemed to unfold with difficulty. The color was darker than that of normally growing plants, indicating that the roots were not permitting the intake of the small amount of calcium that was present. The roots of the plants that grew in the highest amount of aluminum had abscissions at their ends, and to these ends a flocculent precipitate adhered. It would seem that the aluminum was taken in and precipitated and then the degeneration of tissue took place (table 1 and fig. 2).

It is apparent that plants grown in soil or water solutions do take up an appreciable amount of aluminum. Where aluminum is present even in moderate quantities, the plants, especially the roots, contain far more proportionately than is present in a soluble state in the soil.

TABLE 1.—*Growth of tobacco plants as affected by various molar proportions of aluminum citrate and calcium nitrate*

Percentage proportions of 0.006 molar aluminum citrate and calcium nitrate used		Average height and weight of tobacco plants expressed as percentages of the growth in standard solution		Percentage proportions of 0.006 molar aluminum citrate and calcium nitrate used		Average height and weight of tobacco plants expressed as percentages of the growth in standard solution	
Aluminum citrate	Calcium nitrate	Height	Weight	Aluminum citrate	Calcium nitrate	Height	Weight
100	0	23	6	50	50	86	74
95	5	58	34	15	85	95	101
85	15	76	59	5	95	100	105
75	25	76	63	0	100	90	90

Unless other factors obtain, such as the presence of an excessive amount of organic matter or phosphates, a large amount of aluminum occurs with a high concentration of hydrogen ions. It is not clear whether this is the result of hydrolysis of aluminum compounds, or whether a reaction is initiated by the action of hydrogen ions upon the otherwise rather insoluble aluminum compounds. Energy factors bringing about equilibrium of reaction need to be considered.

AMOUNT OF SOLUBLE ALUMINUM IN SOILS IN WHICH TOBACCO, CORN, TIMOTHY, AND CLOVER HAD BEEN GROWN

The aspect of brown root rot of tobacco in soils which are subject to rotation is well recognized in the Connecticut Valley. It is not uncommon to find the disease when tobacco is planted after timothy, clover, or corn, but not on land where tobacco has grown for several years in succession.

Although there is not, to the writer, a rational basis for supposing that different crops increase the amount of available aluminum in the soil, analyses were made of soils of the same type which had grown tobacco, timothy, corn, or clover during the past year. There seemed to be a slight variation in the quantity of soluble aluminum present (table 2), but there was no apparent relationship between the amount of aluminum found in the soils and the occurrence of the root disease.

TABLE 2.—*Parts per million of aluminum in and pH of soils of the same type upon which various crops were grown during the same year*

Crop grown	Aluminum of soil, soluble in water	Aluminum of soil, soluble in 1 percent citric acid	pH value of soil	Crop grown	Aluminum of soil, soluble in water	Aluminum of soil, soluble in 1 percent citric acid	pH value of soil
Corn.....	0	0.2	5.95	Timothy.....	0.3	4.0	5.95
Tobacco.....	4.0	4.5	5.60	Clover.....	.2	.3	5.43

EFFECT OF ALUMINUM ON TOBACCO GROWN IN POTS IN THE GREENHOUSE

The analysis of the soil for aluminum after 2½ months of crop growth indicated that nearly all of the aluminum had been converted from a soluble to an insoluble form in the soil (table 3). The water extract of the soil given originally 100 parts per million of aluminum contained at the time of analysis slightly more than 1 part per million. Phosphate added to this combination formed insoluble aluminum compounds that retarded the formation of ionic aluminum.

A sample of soil in a pot of 1-gallon capacity which had grown tobacco affected by brown root rot was analyzed for aluminum. The pH value of the soil was 5.51. The soil was extracted with a 1-percent acid solution and was found to contain 0.9 part per million of aluminum.

As a result of these analyses the writer finds no relation between the aluminum content of soil solution and the occurrence of brown root rot of tobacco. Tobacco plants grown on the soil to which aluminum sulphate had been added to the extent of 100 parts per million of aluminum showed no deleterious effects. In fact, in some respects the growth was more luxuriant than that of the control plants grown in soil to which no aluminum was added.

TABLE 3.—Parts per million of aluminum in and pH of soils treated with aluminum sulphate and with phosphate, and analyzed after 2½ months of crop growth

Soil no.	Treatment	Aluminum in soil, soluble in water	pH value of soil
1	Aluminum sulphate equivalent to 100 p. p. m. of aluminum.....	1.5	4.40
2	Aluminum sulphate equivalent to 50 p. p. m. of aluminum.....	3	4.56
3	Aluminum sulphate equivalent to 25 p. p. m. of aluminum.....	.2	4.86
4	Aluminum sulphate equivalent to 100 p. p. m. of aluminum, and 2.9 g CaH_2PO_4 per 8 pounds of soil.....	.3	4.55
5	Aluminum sulphate equivalent to 50 p. p. m. of aluminum, and 1.45 g CaH_2PO_4 per 8 pounds of soil.....	.1	5.00
6	Aluminum sulphate equivalent to 25 p. p. m. of aluminum, and 0.73 g CaH_2PO_4 per 8 pounds of soil.....	.2	5.05
7	Nothing added.....	.2	5.05

SUMMARY

Tobacco plants were grown alternately 2 days in a complete nutrient solution and 2 days in solutions containing different percentage proportions of 0.006 M aluminum citrate. Definite toxic symptoms were observed at low concentrations of aluminum. No perceptible increase in rate of growth was observed at low concentrations of aluminum salt.

The presence of phosphates lowers the amounts of ionic aluminum in soil.

No results were obtained that justify the assumption that certain crops give rise to active aluminum when the same soil type is used for their culture.

Tobacco plants were grown in complete nutrient solutions in which the proportions of calcium nitrate and aluminum citrate were varied. The results indicate that the calcium ion may function to some extent to reduce the toxic effects of aluminum. In this group of plants low concentrations of aluminum seemed to increase the growth rate slightly.

DISTRIBUTION AND EFFECT OF PETROLEUM OILS AND KEROSENES IN POTATO, CUCUMBER, TURNIP, BARLEY, AND ONION¹

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INTRODUCTION

Petroleum-oil sprays and fogs are used increasingly to control insect pests of economic plants. These oils are useful in controlling the aphid vectors of potato virus, but it is first necessary to learn which oils are tolerated by potato leaves. Hence, undiluted oils and cresoap emulsions of petroleum oils were tested on potato leaves to determine: (1) The rates of oil penetration into leaves; (2) the symptoms caused by the oils; (3) the oils and their concentrations that potato leaves tolerate without necrosis; and (4) a rapid, easy, accurate method for predicting the probable injury that an oil spray will cause in potato leaves. The purpose of these and other experiments was to discover the distribution and effect of petroleum oils in species of herbaceous plants treated with sprays or undiluted lubricating oils or kerosenes.

REVIEW OF LITERATURE

Besides oil-spray emulsions, there is increasing use of oils dispersed as fogs in air for controlling insects on fruit and vegetables (Herbert (4, 5) and Parker (7, 8)).³ Fungicides, and insecticides other than lead and arsenic, are used with the oils, and this avoids costly troubles with spray residues. Ginsburg (2) described the effects of oils on greenhouse plants, and cited the work of Allen on the use of oils to control potato leaf hoppers. Rohrbaugh (9), illustrated oil penetration in citrus. Knight and Cleveland (6) gave evidence that surface tension and viscosity determine the rate of oil penetration. Chandler, Flint, and Huber (1) reported safe use of 1 percent oil sprays on potato leaves. Young (10, 11) mentioned symptoms of oil injury in potato leaves, and the effects of oils on potato yields; (12) described the use of *Rhizopus* growing in oils to test the toxicity of oils; (13) illustrated freezing phenomena in emulsions of spray oils; (14) described penetration, distribution, and effects of oils in apple leaves; and (15) explained theoretically how oil penetrates protoplasm. Young⁴ described physical phenomena of emulsification and (16) also the conduction of decane from onion leaves to the roots. Young and Morris (17) tested an apple, 22 of the 36 oils described in the present paper.

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² The writer wishes to thank Profs. H. E. Morris and D. B. Swingle of the botany department of Montana State College, for criticizing this manuscript.

³ Reference is made by number (italic) to Literature Cited, p. 933.

⁴ YOUNG, P. A. MICROSCOPICAL OBSERVATIONS ON FORMING AND BREAKING CRESOAP EMULSIONS OF PETROLEUM OILS. Abstract of Papers, Amer. Soc. Plant Physiol., Pittsburgh, Pa., p. 17., December 1934. [Mimeographed.] (Illustrated article in press.)

MATERIALS AND METHODS

The 36 kinds of petroleum oils and kerosenes described in table 1 ranged in viscosity from 31 to 410 seconds, and had sulphonatable residues ranging from 0 to 53 percent. These oils were tested on vegetables from 1928 to 1933, inclusive.

TABLE 1.—*Sulphonatable residues of, and injuries to potato leaves by, the oils used*

Oil or kerosene no.	Name of oil or kerosene ¹	Injury factor ²	Sulpho-natable residue ³	Viscosity	Plants ⁴
			Percent	Seconds	Number
1	St. 13604 R.100P.....	3.4	25	100	4
2	St. 13605 R.CRE.....	3.3	44	220	4
3	St. O. T. 13608 R.....	1.5	5	100	16
4	St. M. S. 13607 R.....	1.8	10	50	12
5	St. 5, 14510 R.....	3.6	41.6	110	4
6	St. Diamond paraffin.....	3.2	High	99	4
7	St. Atlantic Red.....	3.4	High	244	4
8	St. 410 red engine.....	3.8	53	410	5
11	St. gas oil.....	3.9	40	38	9
12	Shell Brown neutral.....	3.4	27	115	9
13	Shell RL99, 70P.....	3.7	31.2	75	12
14	Shell 1.....	3.9	28.5	63	4
15	Shell 2.....	3.4	26.4	58	5
16	Shell 3.....	2.4	10	55	15
17	Shell 4.....	3.4	28.3	108	3
18	Shell 5.....	2.4	9.8	108	4
19	Shell 6.....	3.7	34	219	4
20	Shell 105.....	1.2	2	90	5
21	Shell 106 (SO ₂).....	3.2	15.6	53	3
22	Shell 107.....	1.2	2	51	10
24	N. Amalie 10680P.....	1	0	67	29
25	St. 6, 14819R.....	3.0	10	73	3
26	Shell 198.....	3.2	20	60	3
27	Shell 7, 14776R.....	3.8	43	100	8
31	Stanco Nujol.....	1.2	0	220	6
33	Shell E512 (SO ₂).....	2.9	12.8	52	5
34	Shell E513.....	2.7	13.3	51	5
35	Shell E514 (SO ₂).....	3.2	13.7	74	5
36	Shell E515.....	2.5	14	75	5
37	Shell E516.....	1.7	2.3	65	5
41	St. kerosene 20877.....	1.7	4	31	8
42	St. kerosene 20878.....	3.7	19	31	8
43	St. kerosene 20879.....	3.8	22	31	8
44	Shell kerosene.....	2.5	1	31	9
45do.....	2.4	4.1	31	9
46do.....	3.7	16	31	9

¹ Company name and number of oils and kerosenes. Symbols signify: L. Sonneborn Sons, Inc., New York; Stanco, Stanco, Inc.; St., Standard Oil Co. (Calif.); Shell, Shell Oil Co.

² 1=Necrosis only in heavily oiled spots within 11 days. 2=Necrosis of heavily oiled spots in 1 day and of many lightly oiled spots in 11 days. 3=Necrosis and wilting of heavily oiled spots within 1.5 to 7 hours.

³ Percentage of sulphonatable residue in each oil as determined by the oil companies, except oils 5, 13, 15, 16, and 21 by Green (3).

⁴ Number of potato plants on the leaves of which sufficient oil was placed to make 20 to 60 percent of the laminae translucent.

The effect of oil on the yield of potatoes was tested by spraying cresoap emulsions of oil 4 on potato leaves in a field (table 2). The leaves were drenched with the oils on July 9 and 24, 1931. The potatoes were grown in alternating units of 6 sprayed and 6 unsprayed hills. Only one seed piece was planted in each hill. The hills were 20 inches apart in rows 36 inches apart, so there were 8,712 hills per acre. Each yield was calculated in bushels per acre by multiplying the total yield in pounds by the constant, 145.2, and dividing this product by the number of plants producing the total yield.

The oils were emulsified in water with ammonia-casein and cresoap emulsifiers as described by Young and Morris (17). The cresoap was made by boiling the cresols and soaps together during 3 days.

Oils 3, 4, 13, 21, 22, 24, 36, and 37 in concentrations of 1, 2, 4, 8, and 16 percent emulsified with cresoap or ammonia-casein, were sprayed on leaves of potatoes growing in an experimental plot. The oils described in table 1 were placed in undiluted form on the leaves of Triumph, Russet Burbank, Irish Cobbler, and Idaho Rural potatoes in a greenhouse and an experimental plot.

Oil 3 was placed on the cotyledons of squash and cucumber seedlings, and oil 24 on the leaves of rutabaga and turnip. Seedlings of barley treated with oils 3, 13, 21, and 24, were supplied by Green (3).

Onions also were used in studying oil distribution. Bulbs of Yellow Danvers, Bermuda Yellow, and Spanish Yellow onions were set with their bases in water until they produced new roots and leaves. The leaves, bulbs, and roots were treated with oils 3 and 24. Normal onions were studied for comparison. Oil 24 was injected hypodermically into hollow leaves of onions growing in root-study boxes.

Free-hand sections of fresh, living tissues mounted in distilled water were studied microscopically to determine the distribution and conducting channels of oils in plants. Some samples of oils 3, 13, 16, and 24 had nearly 0.2 percent oil Red O dissolved in them to make the oils easily visible in tissues. Sections containing unstained oils were stained with Sudan IV (14).

EXPERIMENTAL DATA

EFFECTS OF OIL ON POTATO YIELDS

The potatoes sprayed with a Mineral Seal oil 4 in a concentration of 1 percent yielded slightly more than the unsprayed potatoes growing in alternate units (table 2). In contrast, the emulsion of 2-percent oil reduced the yield of the potatoes 9.3 percent (table 2). This decrease in yield probably is not very significant when it is compared with the increase of 4.6 percent in the yield of the potatoes sprayed with the emulsion of 1-percent oil, while growing in the same plot. Hence a spray of 1 percent of oil 4 probably has commercial value because it is expected to control aphids without decreasing the yield of the potatoes. A spray with 2 percent of oil 4 is not recommended because it may cause some injury to potato leaves.

TABLE 2.—*Effects of sprays with cresoap emulsions of oil 4 on yields of Bliss Triumph potatoes at Bozeman, Mont., 1931*

Treatment of potato plants	Plants	Yield of tubers	Calculated yield per acre	Increase (+) or reduction (—) in yield as affected by spray
	Number	Pounds	Bushels	Percent
1-percent oil emulsion.....	55	48.76	128.7	+4.6
Unsprayed.....	58	49.13	123.0	
2-percent oil emulsion.....	103	98.26	138.5	-9.3
Unsprayed.....	109	114.64	152.7	

OIL PENETRATION INTO POTATO LEAFLETS

Hypophyllous applications of the oils to normal Bliss Triumph potato leaves at 20° to 25° C. revealed the following rates of penetration causing immediate translucence: 0.5 to 10 seconds for the kerosenes with viscosities near 31 seconds; 1 to 30 seconds for the oils with viscosities of 50 to 108 seconds; 60 seconds for the oils with viscosities near 220 seconds; and nearly 5 minutes for oil 8 with a viscosity of 410 seconds. The red-stained oils made potato leaves and their stems red during 1 or more weeks. Emulsions containing oils in concentrations of 4, 8, and 16 percent also made oily translucent spots in potato leaves. Similar reactions were observed when oil penetrated the leaves of turnip, rutabaga, onion, and barley, and the cotyledons of cucumber and squash.

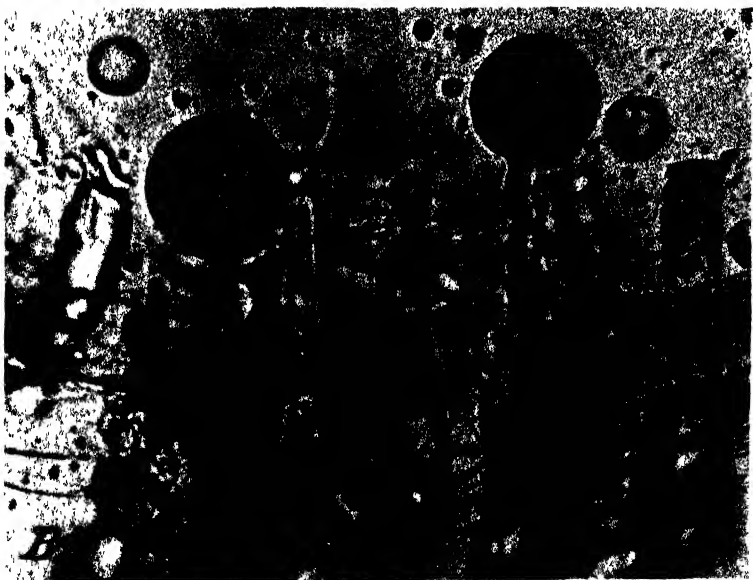
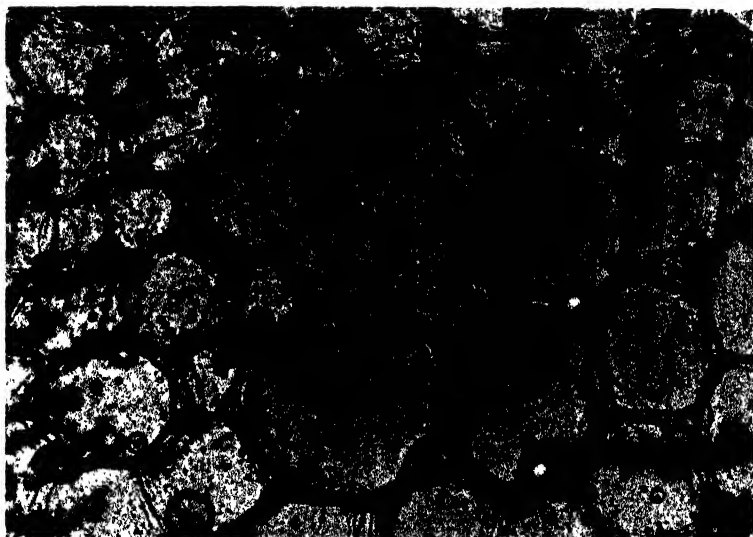
The oily spots in potato and apple leaves were similar in their refraction and reflection of light (14). Potato leaves showing oily spots were stored in the herbarium, where many of the spots retained their oily appearance in the dried leaves during 1 or more years, thus resembling apple leaves in their retention of oil.

DISTRIBUTION OF OILS IN POTATO, CUCUMBER, TURNIP, BARLEY, AND ONION

Oils 3 and 24, with and without Oil Red O stain, were placed on the leaves of potato, turnip, rutabaga, and barley, and on the cotyledons of cucumber and squash seedlings. They were also injected hypodermically into the hollow leaves of onions. The stems attached to the oiled leaves usually became oily 1 to 5 days after the leaves or cotyledons were oiled. The red-stained oils made the tissues red. Sections of such plants were cut to determine the distribution of the oils. The stained and unstained oils were similar in their distribution and effect. Examples of sections showing typical distribution of oils are given in table 3. The oils were abundant between parenchyma cells (pl. 1). Less oil was seen in tracheae and parenchyma cells, so the oil evidently passed mostly between parenchyma cells in being carried from the leaves into the stems and roots of these herbaceous plants. The pneumatic and hydrostatic system of the plant is concerned with the conduction of oil. Oil penetration into parenchyma cells was explained by Young (15).

Red-stained oil 24 passed from Irish Cobbler potato leaves into the new tubers in the soil, making prominent red regions within 1 cm from the point of attachment of the stolons. By forcing a wire through the pith, vertical holes 0.3 cm in diameter and 12 cm deep were made in 4 large stems of a Russet Burbank potato plant, and 1 cc of oil 24 saturated with oil Red O was placed in each hole. The oil was found in 3 of the 9 attached tubers, 8 days later. The petroleum oil in potato tubers was mainly between the starch-parenchyma cells.

Stems were placed in emulsions to study absorption of oils from emulsions, and to determine the toxicity of emulsion cream. Excised potato stems bearing leaves were stood in jars containing cresoap emulsions of 1 and 4 percent oil 24 with oil Red O. Parts 1 to 2 cm long wilted where these stems passed through the cream layers 1 mm thick. In contrast, the leaves and the parts of the stems above and below the cream layers remained alive for a few days. Flowering peduncles of *Oxalis cernua* Thunb. were stood in cresoap emulsions of 1, 2, 4, and 8 percent concentrations of oils 16 and 24 (with and with-



A, Cross section of the seeding stem of a White Spine cucumber showing the globules of oil (photographed black) above the intercellular spaces of the parenchyma cells. The oil had exuded from the polyhedral tubes between the parenchyma cells. Oil 3 stained with Sudan III was placed on the lower sides of the cotyledons of this cucumber on December 3, 1929. The attached stem was frozen in ice and cut 15 days later. $\times 160$. *B*, Like *A*, but an oblique section of this cucumber stem showing the polyhedral, inter-parenchyma tubes bearing globules of oil 3 that exuded from them when they were cut. $\times 633$.

out oil Red O). The parts of the oxalis peduncles passing through the cream layers wilted within 1 to 2 days, while the flowers and the parts of the peduncles below and above the cream layers remained apparently normal during a few days. In contrast, oxalis peduncles standing in water alone, and in water bearing a layer of oil 3 with oil Red O, showed no wilting for several days. Onion leaves were stood in jars containing cresoap emulsions of 1 and 2 percent oil 24 with oil Red O. The parts of the leaves near the cream layers wilted within 9 to 24 hours, while above the cream layers, the parts of the leaves remained turgid.

TABLE 3.—*Distribution of oils in tissues after oils were placed on the leaves of potato, turnip, rutabaga, onion, and barley, and on the cotyledons of cucumber and squash*

Plant	Oil no.	Tissue sectioned	Period between oiling and sectioning	Location of oil ¹		
				Between paren- chyma cells	Inside paren- chyma cells	Inside tracheae
			<i>Days</i>			
Potato	24	Mesophyll	12	+	0	0
	3	Midrib	23	0	0	0
	3	Petiole	23	+	+	0
	3	do	11	+	0	0
	24	Stem	55	+	+	+
	24	do	27	+	+	+
	24	Aerial tuber	39	+	0	+
	3	Roots	11	+	+	+
	3	do	23	+	+	0
	24	Old seed tuber	38	+	+	0
	3	Stolon	11	+	+	0
	24	New tubers in soil	38	+	+	0
Cucumber	24	do	73	+	0	0
	3	Stem	14	+	0	0
	3	do	7	+	+	0
	3	Tap root	7	+	+	0
Squash	3	Fibrous roots	16	+	0	0
	3	Stem	14	+	0	+
Turnip	24	Petiole	23	+	0	0
	24	Root	8	+	0	+
Rutabaga	24	do	9	+	0	0
	24	Petiole	9	+	0	+
Onion	24	Leaf base	2	+	0	0
	24	Root	2	+	0	+
Barley	24	do	20	+	0	+
	21	Leaves	5	+	+	+
	3	Stems	4	+	0	0
	13	Roots	5	+	0	+

¹ + = oil present; 0 = oil absent.

Oil 24 was only slightly toxic while cresoap was very toxic (Young and Morris, 16). Stems wilted where they passed through layers of emulsion cream, which is evidence that abundant cresoap in the cream penetrated the stems. Although wilting occurred in the parts of stems touching cream, the tracheae in the wilted parts continued to conduct water, so the tops of the stems remained alive and turgid for a few days.

Oil 3 saturated with Sudan III was placed on the cotyledons of seedling cucumbers (*Cucumis sativus* L.) variety White Spine, and seedling squashes (*Cucurbita maxima* Duch.). The oil made the cucumber and squash cotyledons red and oily translucent within 10 seconds. The attached stems also became red and oily translucent within 1 to 14 days. Sections of these stems showed the oil mainly between the

cortical parenchyma cells (pl. 1; table 3). These sections containing oil prominently showed the concave-sided polyhedral tubes between the cortical parenchyma cells.

Sections of cucumber and squash stems not attached to oiled cotyledons showed air bubbles in the polyhedral spaces between the parenchyma cells. No oil was seen in these stems.

Oil 24 with oil Red O made leaves of turnip (*Brassica rapa* L.) and rutabaga (*Brassica napobrassica* Mill.) red and oily translucent. The attached petioles and stems also became red and oily within 1 to 8 days. The attached fleshy turnip and rutabaga taproots were sliced 8 days after the leaves were oiled. Large parts of the tissues were red because of the red oil in them, in contrast to the nonoily parts of the same roots (pl. 2, A). Microscopic study of thin sections of stems and roots revealed the oil between the parenchyma cells (table 3).

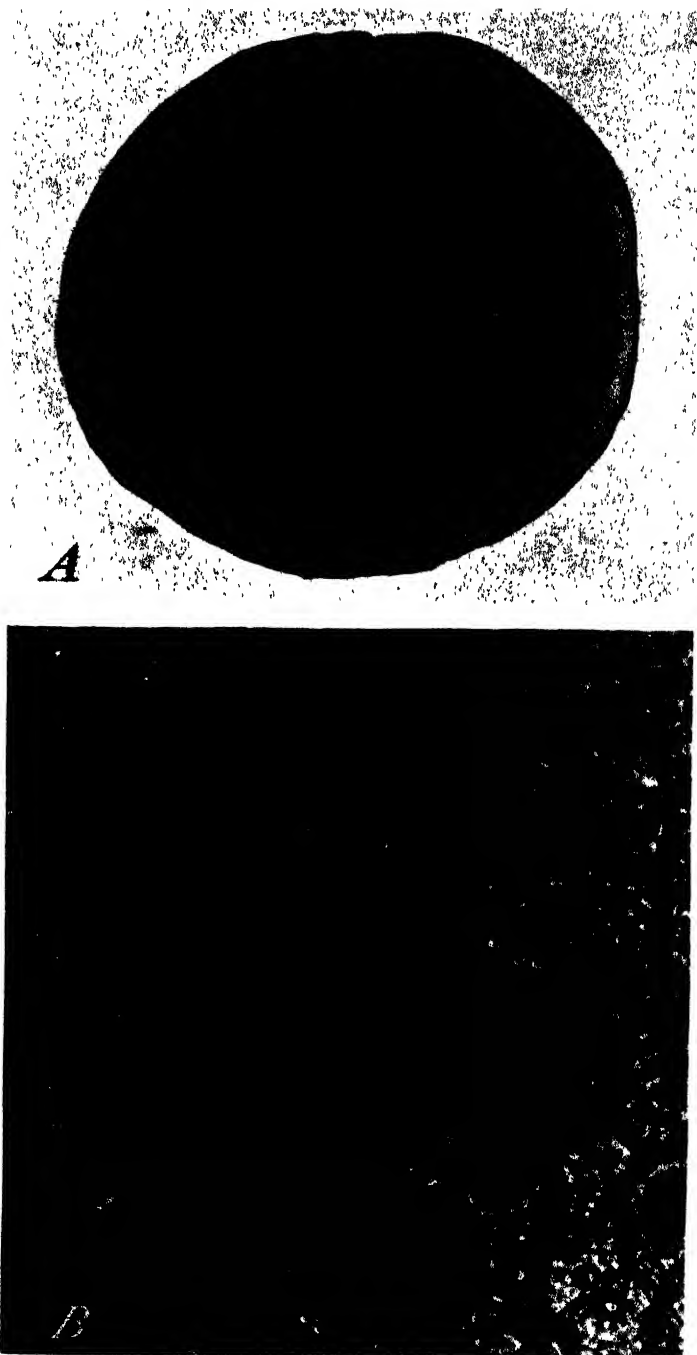
Leaves of barley seedlings (*Hordeum vulgare* L.) turned red and oily translucent within a few seconds after the red-stained oils 3, 13, and 24 were placed on the leaves. The attached stems and roots also turned red within 4 days. Sections of these stems and roots showed oil between and inside the cells (table 2). Oil was seen between collenchyma cells in a stem. Oil 21 was placed on seedling leaves of barley. Sections of the oiled leaves were cut 5 days later and were stained with Sudan IV. The oil was abundant between the chlorenchyma cells, and in the tracheae of a large vein.

Five bulbs of onion (*Allium cepa* L.) were set in root-study boxes. When the new leaves were 5 to 25 cm long, 1 to 4 cc of red-stained oil 24 were injected hypodermically into the tip of each of 1 to 4 hollow leaves of each bulb. During 3 to 9 days, these hollow leaves were oil reservoirs that exhibited the columns of oil by transmitted light. Thirty to ninety percent of the attached roots turned red within 2 to 10 days after the oil was injected into the leaves. Sections of the roots and bulbs showed the oil in their tissues (table 3 pl. 2, B and 3).

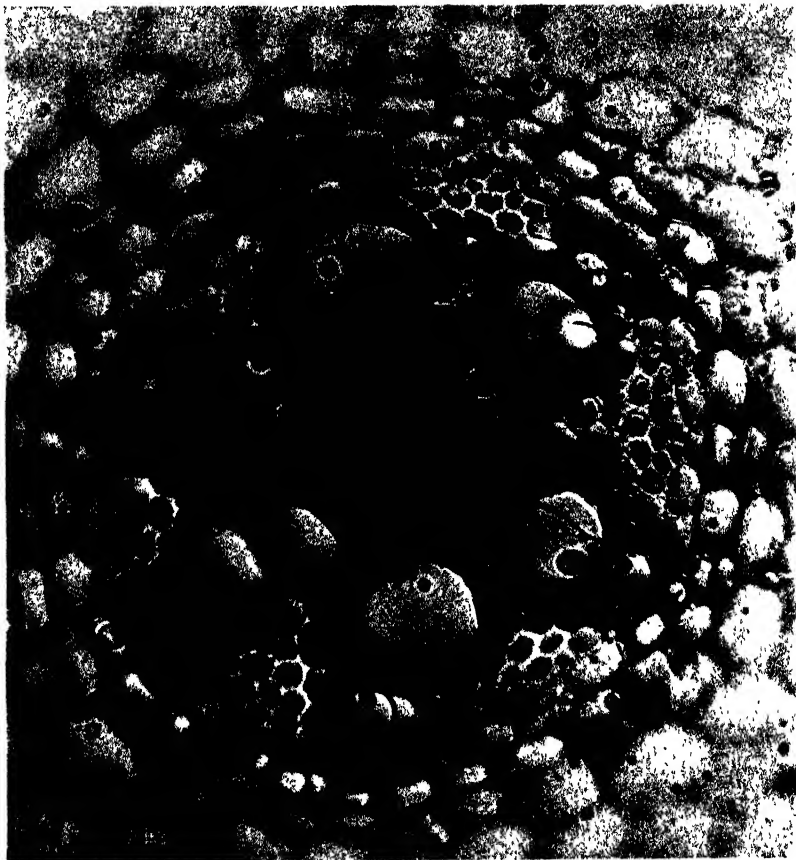
Oil 24 saturated with indophenol blue was injected into the hollow leaves of four other onion bulbs. The attached roots did not change color so some of them were sectioned and stained with Sudan IV, which revealed the oil in and between the cortical parenchyma cells. Indophenol blue greatly increased the toxicity of oil 24 in contrast to oil Red O that apparently did not increase the toxicity of this oil.

The roots of three onion bulbs were placed in jars of water bearing layers of oil 24 with oil Red O. The oil entered many of these roots and was distributed in the attached leaves and bulbs. Two onions were set so that the bulbs were 6 cm above the water into which their roots projected. Oil 24 with oil Red O was placed on the aerial parts of their roots. The oil entered many of these roots and was conducted into the bulbs.

Entrance of oil through wounds was studied by excising the roots on onion bulbs and setting the bulbs in shallow layers of oils 3 or 24 with oil Red O. The bulbs became red within 20 hours. Sections showed oil in the tracheae of a flattened bulb-stem, because oil entered through the cut base of this stem. Also, excised onion leaves remained alive and turgid during 1 to 3 days while they stood with their cut ends in oil 24 with oil Red O. Much oil entered these leaves. No oil was seen in any of the check onions studied for comparison with the onions treated with oils.



A, Oil 24 stained with oil Red O (photographed black) in a cross section of a rutabaga root 8 days after this oil had been placed on the attached leaves; much of the oil had passed from the leaves into the fleshy root. $\times 1$. B, Oil 5 stained with oil Red O (photographed black) occupying the vertical interparenchyma pockets in the solid part of a second-year onion stem inside the bulb. This cross section was cut after the base of the bulb and the upper parts of the roots had touched the oil during 11 days. $\times 200$.



Oil 24 stained with oil Red O (photographed black) in the central trachea of a root of a Sweet Spanish onion. The oil in this root came from one attached hollow leaf into which 3.5 cc of oil were injected hypodermically 20 days previously; 13 of the roots became red within 2 days. $\times 305$.

The roots of six onion bulbs were arranged so that half of the roots on each bulb projected into water alone, while the other roots projected into test tubes of water bearing layers 1 cm thick of oils 3 or 24 with oil Red O. The oil entered the roots that had wounds in their parts in the oil layers. Branch roots naturally make wounds as they rupture their way through the cortex. The sides of the bulbs turned red above the oiled roots. Also some of the other roots turned red within 3 days, although they projected into water alone. Sections cut 2 days later showed oil in the tracheae and parenchyma cells of these roots. A small aerial leaf on one bulb turned red. Sections of it showed oil between the chlorenchyma cells. Thus, oil was conducted from the roots into this leaf. Sections of the red parts of these bulbs showed oil between the parenchyma cells of the fleshy bulb scales (bases of first-year leaves). Also the cylindrical stems in the bulbs (bases of the second-year leaves) showed abundant oil between the parenchyma cells, especially inside the vertical interparenchyma gas pockets (pl. 2, *B*).

Oil 24 with oil Red O facilitated determination of the nature and function of the large interparenchyma pockets (pl. 2, *B*). These apparently vertical pockets were surrounded by specialized parenchyma cells. The interparenchyma pockets abounded in the leaves and in the cylindrical stems inside and above the bulbs. Sections showed that these pockets naturally contained gas, but were filled with oil in many of the onions treated with oils. These pockets were not seen in very small leaves and their stems inside onion bulbs, but developed as the leaves and stems matured. None of these gas pockets was seen in the fleshy bulb scales, nor in the flattened bulb stems. These flattened bulb stems showed parenchyma cells with ramifying tracheae that usually did not contain petroleum oil. The interparenchyma gas pockets that were centrally situated in maturing onion leaves showed ruptured walls. Evidently many of the pockets united to form the hollow centers of the maturing leaves. These interparenchyma pockets are part of the hollow mechanical structure of onion leaves.

SYMPTOMS CAUSED BY LUBRICATING OILS AND KEROSENES IN POTATO LEAFLETS

Undiluted lubricating oils placed on potato leaflets often caused wilting preceding necrosis in oily translucent leaflets. Some of the oiled juvenile leaflets rolled upward. They were more susceptible to oil injury than were mature leaflets. Sprays of lubricating petroleum oils in concentrations of 4, 8, 16, and 100 percent commonly caused black and brown spots in potato leaflets. Oils often accumulated over epiphyllous veins and blackened them. Large amounts of toxic oils killed the leaves and stems. Sprays with emulsions containing 2 percent of toxic oils often caused brown spots 1 to 10 mm wide in leaflets.

Oil 21 caused purpling in some leaflets. Probably the other oils lacked sufficient concentration of the chemicals that caused purpling in potato leaflets.

Epiphyllous white and light-gray spots appeared in many potato leaflets treated with 4-, 8-, and 100-percent concentrations of oils 4, 7, 13, 21, 27, 42, 43, and 45. Most of these white spots were hypophyllously green, brown, or dark gray. Epiphyllous white spots served as a useful symptom in distinguishing these oils. Probably

only these oils contained the casual chemicals in concentrations sufficient to cause white leaf spots. Oils 13 and 21 caused white spots abundantly and regularly, so that prominent production of this symptom distinguished oils too toxic for use in sprays on potato leaves.

The translucence caused by kerosenes usually disappeared within 1 to 24 hours, as the kerosenes evaporated from the intercellular spaces. The kerosenes caused chlorosis, brown spotting, marginal rolling, and blackening and drying of the rolled parts. The petioles died on many of the badly injured leaves.

Oils 41, 44, and 45 caused no injuries or only mild injuries in potato leaves, while oils 42, 43, and 46 caused wilting within 2 hours in heavily oiled leaflets, and killed many of the petioles (table 1).

INJURIES CAUSED BY PETROLEUM OILS IN POTATO LEAVES

In table 1, the physical suffocating effect of the oils presumably caused injuries represented by the injury factor 1, as oil 24 did, whereas additional injuries represented by larger injury factors such as 1.2 to 3.9 presumably include the chemical effects of toxic compounds in the oils, according to a critical definition of toxicity (14). Oils 14, 15, 44, and 46 were more injurious than their percentages of sulphonatable residues indicated, probably because some of the sulphonatable chemicals were especially toxic to potato leaves. Usually, the kerosenes and lubricating oils with low percentages of sulphonatable residues caused only slight injuries, even when they made the laminae translucent. In contrast, the more sulphonatable oils caused serious necrotic symptoms even when they were applied in concentrations of only 2 percent.

Practical conclusions are drawn from table 1. The toxic effects of oils in potato leaves were tested accurately, easily, and quickly by placing drops of each undiluted oil on normal potato leaves at temperatures below 35° C., and before the leaves show senescence. The rapidity, severity, and abundance with which the symptoms appeared showed the toxicity of each oil to potato leaves. The oils that caused amounts of injury approximately indicated by the injury factor 3 presumably are too injurious for sprays on potato leaves. The oils that caused amounts of injuries indicated by the injury factor 2 probably are too toxic to use in emulsions containing more than 1 percent of oil. The oils that caused amounts of injury indicated by the injury factor 1 are preferable for sprays in concentrations of 1 and 2 percent of oil on potato leaves.

SUMMARY

Cresoap emulsion of 1-percent oil 4 did not reduce the yield of potatoes, while an emulsion of 2-percent oil caused a 9.3 percent reduction in the yield.

Lubricating oils caused the following symptoms in potato leaflets: Translucence, wilting, rolling, chlorosis, blackening and browning of veins, and spots of laminae; also purpling and white leaf spots. Only the very toxic oils caused severe injuries including purpling and white leaf spots.

Kerosenes caused the following symptoms in potato leaves: Temporary translucence, chlorosis, brown leaf spots, marginal rolling with

blackening of the rolled parts, and killing of petioles. Only the very toxic kerosenes caused the severe symptoms.

A convenient method of testing the toxicity of oils and kerosenes before they are sprayed on potato leaves is to place drops of each oil, and drops of unsulphonatable oil, on healthy potato leaves, and observe the comparative symptoms. Oils that cause more than a few necrotic symptoms within the first few days probably are too toxic for commercial sprays on potato leaves.

The oils and kerosenes with low percentages of sulphonatable residues usually caused only slight injuries even when the laminae had been oily-translucent. The slightly toxic oils may be used safely in sprays of 1 percent oil on potato leaves.

Kerosenes penetrated potato leaves within 0.5 to 10 seconds, but mostly evaporated from the leaves within 1 to 24 hours. In contrast, lubricating oils penetrated potato leaves within 1 second to 5 minutes, and usually remained in the leaves during the remainder of the growing season. Sections showed that the oils passed from potato leaves through the stems and into the tubers.

Emulsion cream caused wilting in stems of potato and oxalis, but did not quickly stop the conduction of water through the wilted parts of the stems.

Red-stained oils placed on cucumber and squash cotyledons quickly passed into the stems and made them red. Sections showed the oil mainly between the cells.

The red-stained oils placed on turnip and rutabaga leaves passed into the fleshy tap roots and made large parts of them red. When red-stained oils were placed on barley leaves, they passed through the stems and made the roots red. The oil Red O stain apparently did not affect the toxicity of either oil 3 or 24.

Red-stained oils injected into onion leaves passed through the bulbs and made many of the roots red. Sections showed the oil in the tracheae and between the cortical parenchyma cells. Oils also passed from roots into attached bulbs and leaves. Leaf and stem sections showed oil in the large pockets between the cells. These interparenchyma pockets in leaves, and second-year stems in bulbs, apparently serve as gas chambers.

Study of these vegetables showed that the petroleum oils were mainly between the parenchyma cells, but also were inside tracheae and parenchyma cells. Evidently, the oils are conducted mainly between the cells in passing from leaves into roots.

LITERATURE CITED

- (1) CHANDLER, S. C., FLINT, W. P., and HUBER, L. L.
1926. RECENT INSECTICIDE EXPERIMENTS IN ILLINOIS WITH LUBRICATING OIL EMULSIONS. Ill. Nat. Hist. Survey Bull. 16: [103]-126, illus.
- (2) GINSBURG, J. M., SCHMITT, J. B., and GRANETT, P.
1935. UTILIZATION OF A COMPLETELY REFINED, LOW-BOILING PETROLEUM DISTILLATE IN CONTROLLING INSECTS INFESTING CHRYSANTHEMUM AND OTHER PLANTS. Jour. Econ. Ent. 28: 236-242, illus.
- (3) GREEN, J. R.
1932. CHEMICAL AND PHYSICAL PROPERTIES OF PETROLEUM SPRAY OILS. Jour. Agr. Research 44: 773-787, illus.

-
- (4) HERBERT, F. B.
1933. AIRPLANE LIQUID SPRAYING. *Jour. Econ. Ent.* 26: 1052-1056, illus.
- (5) ———
1934. AIRPLANE VAPOR SPRAYING: A PROGRESS REPORT. *Jour. Econ. Ent.* 27: 1040-1042.
- (6) KNIGHT, H., and CLEVELAND, C. R.
1934. RECENT DEVELOPMENTS IN OIL SPRAYS. *Jour. Econ. Ent.* 27: 269-289, illus.
- (7) PARKER, W. B.
1933. VAPO DUST—A DEVELOPMENT IN SCIENTIFIC PEST CONTROL. *Jour. Econ. Ent.* 26: 718-720, illus.
- (8) ———
1934. RECENT DEVELOPMENTS OF THE VAPO DUST METHOD OF PEST CONTROL. *Jour. Econ. Ent.* 27: 1036-1040.
- (9) ROHRBAUGH, P. W.
1934. PENETRATION AND ACCUMULATION OF PETROLEUM SPRAY OILS IN THE LEAVES, TWIGS, AND FRUIT OF CITRUS TREES. *Plant Physiol.* 9: 699-730, illus.
- (10) YOUNG, P. A.
1931. PENETRATION AND TOXICITIES OF PETROLEUM-OIL SPRAYS. (Abstract) *Phytopathology* 21: 130.
- (11) ———
1932. LUBRICATING-OIL SPRAYS AND THEIR EFFECT ON POTATOES. (Abstract) *Phytopathology* 22: 31.
- (12) ———
1934. FUNGI AND BACTERIA AS INDICATORS OF THE EFFECTS OF PETROLEUM OILS ON APPLE LEAVES. *Phytopathology* 24: 266-275, illus.
- (13) ———
1934. FREEZING PHENOMENA IN CRESOAP EMULSIONS OF PETROLEUM OILS. *Plant Physiol.* 9: 795-804, illus.
- (14) ———
1934. PENETRATION, DISTRIBUTION, AND EFFECT OF PETROLEUM OILS IN APPLE. *Jour. Agr. Research* 49: 559-571, illus.
- (15) ———
1935. OIL-MASS THEORY OF PETROLEUM-OIL PENETRATION INTO PROTOPLASM. *Amer. Jour. Bot.* 22: 1-8, illus.
- (16) ———
1935. DECANE RING-SPOT OF APPLE LEAVES, AND SYMPTOMS OF DECANE INJURY IN APPLE, POTATO, AND ONION. *Amer. Jour. Bot.* 22: 629-634, illus.
- (17) ——— and MORRIS, H. E.
1933. INJURY TO APPLE BY PETROLEUM-OIL SPRAYS. *Jour. Agr. Research* 47: 505-522, illus.

THE DEVELOPMENT OF THE COTTON EMBRYO¹

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INTRODUCTION

The development of the cotton embryo was treated briefly from the morphological point of view by Balls (1),² Flatters (2), and Gore (4). None of these investigators, however, made a chemical study of the developing embryo. Ordinarily such investigations have not been made because of the impracticability of separating the immature embryos from the surrounding tissues in sufficient quantities to make satisfactory analyses. Instead of the usual chemical analyses it is often practical to use microchemical tests, for, although they have the disadvantage of not yielding quantitative results, they have the advantage of being applicable to plant tissues in their natural position and to comparatively small quantities of material.

This study of the development of the cotton embryo was undertaken for the purpose of obtaining a knowledge of the rate of growth, anatomical development, and chemical development, as related to each other and to the age of the embryo from the first division of the zygote to dormancy.

METHODS

In July 1934 approximately 150 flowers of an American upland cotton (Startex) were self-pollinated. As the time after pollination that is required for fertilization to occur has been reported by Gore (4) to be from 26 to 32 hours, the present report includes no studies to determine the length of this period, but it is assumed that fertilization occurred within 36 hours. Material was collected, killed in Licent's fluid, sectioned, and stained with haematoxylin or safranin. Collections of embryos were made at the following periods (days) after fertilization, allowing 36 hours for fertilization to be completed: 1, 2, 3, 4, 6, 9, 12, 15, 16, 18, 22, and 26. Before the twenty-sixth day most of the important anatomical developments had occurred, and the oldest of the material was not used for anatomical studies. However, microchemical tests and growth studies were made on the older material.

Some difficulty was experienced in making microchemical studies on fresh material before the sixth day. This was caused by the minuteness of the embryo and the fact that it gave the same reactions as the endosperm surrounding it.

Weights of embryos 16 days of age and older were taken immediately after their dissection from the ovules, and again after drying in air for 24 hours. The lengths of embryos were recorded from the sixth day until maturity.

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² Reference is made by number (italics) to Literature Cited, p. 944.

EARLY DEVELOPMENT OF THE EMBRYO

The first division of the zygote, which ordinarily occurred the second day after fertilization, was usually horizontal (fig. 1, *A*). This was followed by a vertical division of the apical and sometimes of the basal cell (fig. 1, *B*). Occasionally a diagonal division instead of the vertical occurred in the apical cell, giving rise to such a structure as that shown in figure 1, *C*. The exact manner of origin of this configuration was not determined because of its relatively rare occurrence. A second departure from the condition shown in figure 1, *B*, occurred when the basal cell failed to divide (fig. 1, *C*, *D*), resulting in a one-celled suspensor. Three-celled proembryos were most frequently found in ovules killed the second day after fertilization.

An intermediary tier of cells was found (fig. 1, *K*), but never a single intermediary cell, as was reported in *Malva rotundifolia* by Souèges (6). A perfect quadrant was probably formed, and various slight departures from it were common. A group of three cells at the apex (fig. 1, *E*) also was found. The structure shown in figure 1, *E*, occurred in material killed the third day after fertilization.

A short suspensor was formed, but in some cases it showed early signs of disorganization. The latter fact may explain Balls' (1) statement that the cotton embryo has no suspensor.

In material taken the fourth day it was found that octants (fig. 1, *H*) had been produced from the quadrants by further cell division. About the same amount of irregularity was observed in the octants as had been found in the quadrants. Material taken the same day also showed mitoses and periclinal walls separating off the dermatogen (fig. 1, *G* and *I*).

Although the stages found here are irregular, if the succession shown in figure 1, *A*, *B*, *D*, *F*, *H*, and *J* is followed, the history of the development of the cotton embryo will be seen to be similar to that described by Souèges (6) in *Malva rotundifolia*. He gave the following account: The first division is horizontal and is followed by a vertical division. The bipartition of the elements of the tetrad gives an octocellular proembryo possessing four circumaxial cells in its upper part. Sometimes 1 of the 2 juxtaposed elements of the tetrad is segmented horizontally or obliquely. The 4 quadrant cells at the apex are separated by walls which take insertion on the peripheral membrane and come down to the vicinity of the axis on the lower horizontal wall (prenant insertion sur la membrane périphérique et venant tomber au voisinage de l'axe sur la paroi horizontale inférieure). Each of the 8 cells then divides, giving rise to a 16-celled proembryo. The wall of segmentation of the quadrant is often horizontal. Five tiers of cells are produced that give rise wholly or in part to cotyledon, hypocotyl and initials of the central cylinder, cortex at the summit of the radial central portion of the tip, and suspensor. This description of the embryogeny of *Malva rotundifolia* appears to be the only record in the literature relative to the early stages of the developing embryo in any malvaceous plant other than cotton.

A great deal of irregularity was found in the details of the early stages of development in the cotton embryo; however, the stages of development illustrated were studied carefully and to all appearances are natural. No doubt the various embryos were sometimes observed from different sides, but if they were radially symmetrical and regular

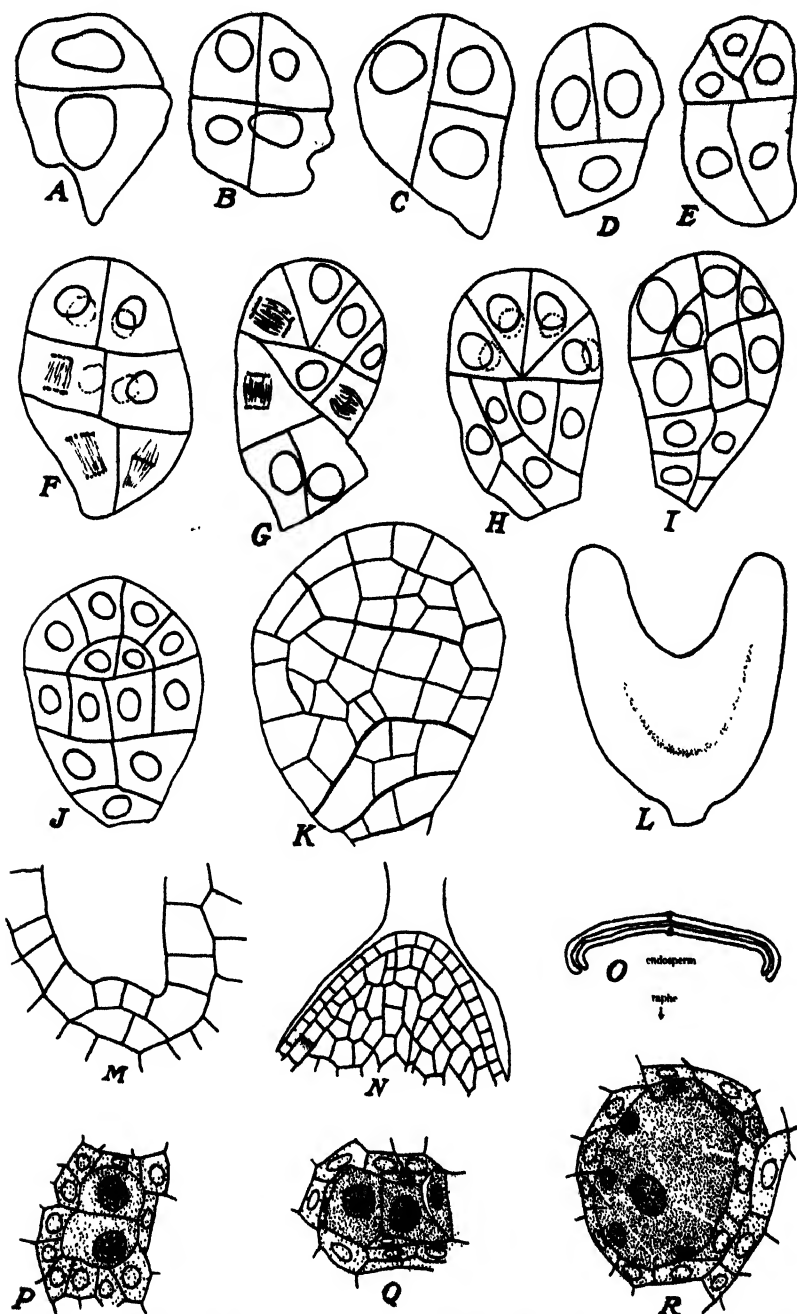


FIGURE 1.—A-K, Stages in development of the cotton proembryo and embryo in approximate succession, including irregular conditions, $\times 550$; L, 9-day-old embryo showing early differentiation of cotyledons, hypocotyl, and histogens, $\times 123$; M, apex of a 9-day-old embryo in a slightly later stage of development, showing the beginning of the plumule, $\times 550$; N, plumule of a 15-day-old embryo, $\times 240$; O, cross section of cotyledons of a 12-day-old embryo, showing their position with reference to the endosperm and raphe $\times 20$; P-R, successive stages in the development of resin glands, $\times 550$.

in early development, as proembryos are often thought to be, their appearance would always be much the same.

Because of the irregularities noted it is impossible to state the exact age at which cotton embryos reach any particular stage of development, but the data given throughout this paper probably represent the conditions that occur most frequently at the ages indicated.

The first microchemical tests were made when the embryo was 6 days of age. At this time, carbohydrates (Molisch reaction) and proteins were present. Negative results were obtained, however, when tests were made for starch, pentosans, and oils. The endosperm, which was very abundant at this age, gave exactly the same reactions as the embryo.

THE GRAND PERIOD OF DEVELOPMENT

The early development of the cotton embryo, as described above, took place before the rapid increase in size of the embryo had begun. About 9 days was required for the initials of the main organs, such as cotyledons, hypocotyl, and plumule, to be formed. The embryo then entered a period of rapid growth, and details of structure continued to manifest themselves as development proceeded. Organization of the cotyledons began between the sixth and ninth day (fig. 1, *L*), when the embryos became heart-shaped in general outline with the two auricles developing into cotyledons and the ventricle end into a hypocotyl.³ Soon after the cotyledons had assumed their form a small mound of tissue could be observed in the axil between them (fig. 1, *M*). This was the first appearance of the plumule as such. The plumule did not become conspicuous until the fifteenth day (fig. 1, *N*).

Between the sixth and ninth day, the histogens of the hypocotyl made their appearance. The limits of the dermatogen, periblem, and plerome were recognized without difficulty on the ninth day. In material of this age proteins and starch were found to be present and pentosans and oil were absent. Reactions in the endosperm indicated a slight amount of starch, but otherwise the same materials were detected there as in the embryo. At 12 days of age the embryos averaged about 1.6 mm in length, and had taken a position on the side of the embryo sac approximately opposite the raphe (fig. 1, *O*).

In embryos 12 days of age the cotyledons averaged about 7 layers of cells in thickness, these layers being the upper and lower epidermis and 5 layers of mesophyll. The cells were fairly uniform in size and shape and often were in regular rows. As the embryos advanced in age the cotyledons increased in thickness by an increase in size and number of cells. At 12 to 15 days of age the earliest signs of provascular strands were found in the cotyledons. These signs appeared first through the formation of cell walls horizontal to the surfaces of the cotyledon, and later by walls vertical to these surfaces and longitudinal to the axis of the provascular strands. Differentiation of the palisade cells of the cotyledons had begun, also, at this age, the second layer of cells from the upper surface being 50 to 100 percent longer than the other mesophyll cells.

³The term "hypocotyl" is used in this paper in the broader sense to include all the organs of the embryo below the attachment of the cotyledons.

In 15-day-old material several other changes were apparent, the most interesting being the first signs of the resin glands. The first indication of a gland was the appearance in the cotyledon of a few (usually 2 or 3) large cells whose protoplasm was very granular and stained rather darkly (fig. 1, *P*). The surrounding cells were of ordinary size and shape except that they were slightly flattened. As the embryo advanced in development, the large cells increased in size, and the cells surrounding them became more flattened (fig. 1, *Q*). Signs of disorganization of the protoplasts of the large cells were obvious on the sixteenth day, for the nuclei had lost their identity, cell walls had begun to disappear, and the contents of the cells had assumed a still more granular, but otherwise homogeneous, appearance. A few layers of the surrounding flat cells also became disorganized and the cells surrounding them in their turn became flat and showed signs of disorganization (fig. 1, *R*). At this age the young glands were well on their way in development, and no structural changes were found between this condition and maturity, except a continuation of the phenomena already described.

Microchemical tests made on embryos 16 days of age showed traces of oil but no pentosans. Reactions of protein with biuret and Millon reagents were more intense in the embryo than in the endosperm, and this difference was observed until the seed was mature.

In embryos 18 days of age, the contents of the young resin glands gave the same color reaction with sulphuric acid that had been obtained in glands of mature embryos. Marchlewski (5) attributed this reaction in the mature embryos to the presence of gossypol. This test is by no means specific for gossypol, but when carefully used it is a fair indication of that substance. Sulphuric acid will produce a red color in other parts of the embryo, but the particular shade of color and the streaming of the gland contents observed at this stage of development are identical only with those obtained in the glands of mature embryos. It may well be emphasized here that the reactions indicating gossypol were not observed at the time of the first appearance of the glands, but only in embryos 18 days old or older. Gallup (3), in his studies of the time of appearance of gossypol in cottonseeds found gossypol first in 32-day-old seeds. His analyses were made on the entire ovule, rather than on the embryo alone as was done in the present investigation. Naturally, gossypol would be relatively less abundant in the entire ovule, and this may account for his not finding it in the young ovules where it had not become plentiful.

A study of 18-day-old material showed that pentosans had appeared in the glands and that the amount of oil in the cotyledons had greatly increased. Starch and proteins were still abundant in both the embryo and endosperm; and since it is well known that proteins, oil, and pentosans are common in fully mature embryos, tests for them were discontinued at 18 days after fertilization. Starch, however, was known to practically disappear before maturity, and in order to determine the time of its disappearance, tests were made for it until this condition was found. Starch seemed to disappear so gradually that the time when it began to decrease was not determined. It was found to be scarce at 33 days after fertilization; and traces were rarely found in seeds that were apparently mature. When the embryo was 18 days of age, all of the organs and provascular tissues were well on their way in development. All important chemical components of

the embryo identified in this study had also begun to appear, and most of them were fairly abundant. The remainder of the period of development of the embryo must therefore consist chiefly of changes (usually increases) in the amounts of the various components and increases in the size of the organs and in the size of the embryo as a whole.

Microchemical tests for sugar in the embryos were never positive except possibly with the Molisch and the Fehling tests. Tests for sugars were made not only on the embryo but also on young lint hairs and the seed coat. The results of these tests are given in table 1. From this table it may be seen that glucose is present in the developing lint hairs. The results were checked and found identical with results obtained on known corresponding substances. Although tests for glucose, except in the form of glucosides, conducted by other investigators on all parts of the cottonseed have consistently given negative results, there can be no doubt that the results obtained in this study on the young lint hairs are typical glucose reactions. In addition to the results summarized in table 1, tests for glucose were made on lint hairs soon after their appearance and also in the later stages of development. The results showed that glucose is an important component of the lint hairs from the time of their first appearance until the boll begins to open. The occurrence of glucose in these hairs is not surprising in view of the fact that the hairs are composed chiefly of cellulose and that a close relationship is known to exist between glucose and cellulose.

Positive reactions for glucose and fructose were obtained on the seed coat during its development, although these results were somewhat masked by a flocculent precipitate of some other component. After the reactions had been carefully studied and the tests repeated on filtered extracts of the seed coats, it was concluded that this structure contained substances that gave the reactions of fructose and glucose. The glucose reaction was not as strong as that of fructose.

TABLE 1.—*Summarized results of microchemical tests for sugars in the cottonseed coat, embryo, and lint hairs 10 to 26 days after fertilization*

Test	Lint hairs	Seed coat	Embryo
Phenylhydrazine.....	Positive, glucose.....	Positive? glucose.....	Negative.
Flückiger.....	do.....	Positive? glucose and fructose.....	Negative?
Fehling.....	Positive.....	Positive.....	Positive?
Molisch.....	do.....	do.....	Do.

Embryos gave the Molisch reaction for carbohydrates from their early formation to maturity; however, the Flückiger and phenylhydrazine tests showed that this was not the result of the presence of glucose or fructose. Several other sugars and glucosides are known to occur in mature cotton embryos, and probably one of them was the substance that gave the reaction for carbohydrates. Indeed, starch, which is known to be present in developing embryos, gives positive reactions with the Molisch reagent, but it usually reacts more slowly than the carbohydrate under investigation here. The Fehling test showed a somewhat doubtful reaction for reducing sugars. A precipitate occurred, but its appearance was not typical of the copper oxide that precipitates when the test is applied to any of the common sugars.

THE CLOSING PERIOD OF GROWTH

In the closing period of growth of the embryo there were fewer changes than in the grand period. Storage materials continued to form, so that there was a slow increase in dry weight, but there was a great loss of water and therefore a loss in total weight. No new tissues or organs appeared and no new chemical components were recognized.

THE GROWTH CURVE

For several decades biologists have recognized the fact that the normal growth curve of an organism takes the general form of a somewhat straightened S; that is, growth begins slowly (the formative period), later becomes much more rapid (the grand period), and then again becomes slow preparatory to cessation (the maturation period). In the life of the sporophyte of a cotton plant, there are at least two such growth curves.

The first represents the development of the embryo from the zygote to the mature seed in the resting period, and the second represents the time from the awakening of the embryo from its dormant period until the sporophyte has reached maturity. In the cotton embryo, the exact form of the growth curve (figs. 2 and 3) was found to vary in some details, depending upon whether the size of the embryo was recorded as length, immediate weight, or air-dry weight. The curves

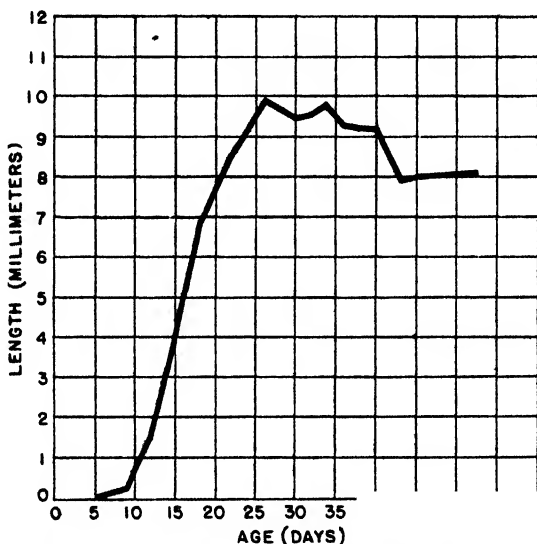


FIGURE 2.—Length of cotton embryos at different ages.

are very similar, however, and the differences are easily explained. It was not found practical to weigh embryos until 16 days after fertilization, but measurements of length were taken as early as 6 days after fertilization. The embryo entered upon its grand period of development in length about a week before it entered this period of development in weight. This is explained by the fact that during early development length increases more rapidly than volume. On the fifteenth day the embryos were approximately 3.8 mm long. Then they began to expand laterally, filling the cavity of the growing seed coat. From that time until the seed reached its greatest length, the weight and length curves were very similar; however, when this period was reached, about the twenty-sixth day after fertilization, the weights continued to increase at the same rate. The last 3 days of this period make up the period of maturation. The embryos continued to increase in actual size (live weight) until the thirty-fourth or thirty-fifth day, when they began to lose weight by drying. They

reached an equilibrium between the forty-third and forty-seventh day and then remained constant. The period of maturation in live weight occupied from the thirtieth to the thirty-fourth day. In air-

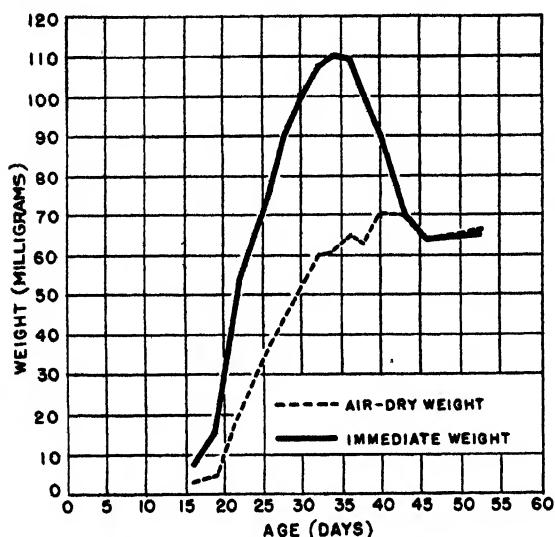


FIGURE 3.—Weight of cotton embryos at different ages.

dry weight, the embryos increased steadily until the thirty-second day and then entered upon the period of maturation. They attained their greatest weight on the fortieth day, although the variation observed in the curve after the thirty-sixth day was probably caused by the smallness of the samples. The curves based on weights show the period of maturation more adequately than the curve based on length. Gallup (3) states that the weight of the embryo may be expected to decrease slightly after maturity, possibly through respiration. A decrease would naturally occur in any seed, but the amount of decrease would be negligible.

The principal events observed in the developing embryos with their corresponding ages are summarized in table 2.

TABLE 2.—Age in days of cotton embryos with corresponding morphological and chemical changes, green weight, dry weight, and length

Morphological and chemical changes	Age	Embryos weighed	Green weight	Dry weight	Embryos measured	Length
	Days	Number	Mg	Mg	Number	Mm
Occasionally first division of zygote.....	1					
First or second division of zygote.....	2					
Quadrant and other similar stages.....	3					
Octant; separation of dermatogen.....	4					
Carbohydrates (Molisch reaction) and proteins present.....	6				2	0.07
Early organization of cotyledons and hypocotyl; appearance of histogens and starch.....	9				2	0.29
Location of embryo at side of embryo sac opposite raphe.....	12				15	1.6
Appearance in cotyledons of provascular strands, resin glands, and palisade tissue; early differentiation of plumule, appearance of oil.....	15 16				16	3.8
	16	20	7.5	3.0		
Appearance of gossypol and pentosans.....	18				13	6.8
	19	14	16.4	4.6		
	22	14	53.6	21.0	5	8.0
	26	20	77.8	38.0	12	9.9
	30	55	100.2	52.0	30	9.5
	32	14	127.1	60.0	29	9.6
	34	20	110.3	61.2	30	9.8
Continuation of growth and differentiation of tissues that had already arisen.....	36	59	109.8	64.7	31	9.3
	38	76	99.4	63.0	76	9.2
	40	50	90.6	70.2	50	9.2
	43	44	70.2	66.5	44	7.9
	46	30	83.5	63.3	30	8.0
	53	20	65.7	65.9	20	8.1

ABNORMALITIES AND IRREGULARITIES

Although the material used in this study was thought to be a relatively uniform race of American upland cotton, much irregularity was observed in the size of embryos of identical ages.

A few examples will suffice to show the range in size of embryos of the same age. Three 29-day-old bolls were taken at random, embryos were dissected from them, and the average live weights were found to be as follows: Boll 1, 20 embryos averaged 72 mg; boll 2, 20 embryos averaged 111 mg; and boll 3, 15 embryos averaged 123 mg.

After the bolls had been dried in air until an equilibrium was reached, the following average weights were recorded: Boll 1, 20 embryos averaged 33 mg; boll 2, 20 embryos averaged 60 mg; and boll 3, 15 embryos averaged 63 mg. It should be emphasized that these figures are the averages of 15 or 20 embryos, taken at random from the boll, and that the variation among individual seeds was probably much greater than that shown here.

Among the embryos from a single 19-day-old boll the range in length was from 3.5 to 7 mm. If this range were stated in volume or weight it would be much greater. In a fully open, 53-day-old boll, the range in length of 10 embryos taken at random was from 6 to 9 mm. This was an ordinary sized boll having 5 good locks and 37 seeds.

Such cases of variation were not at all uncommon, although it cannot be said that they were of regular occurrence. Whenever so wide a range of variation occurred it was usually in part the result of the fact that a few embryos were much smaller than others. For example, in the last case cited above, the individual measurements in millimeters were as follows: 6, 7.5, 8, 8, 8, 8, 8, 8.5, 8.5, and 9. Numerous ovules of all ages were found which at first appeared to be entirely devoid of embryos, but which upon close examination showed small, sickly ones.

Ovules that showed no embryo sacs, or very abnormal ones, were found occasionally. Undeveloped ovules caused by a lack of fertilization were common, of course, but were not among the irregularities studied. Observations indicated that the position of the seed in the locule was related to its ultimate size. Position is undoubtedly related to shape of embryo and therefore to length. Rea (7) obtained some evidence that motes, or abortive ovules, are caused by a lack of fertilization. Some of the irregularities reported here are undoubtedly related to motes, and further embryological studies should reveal useful information as to how they originate.

SUMMARY

A study was made of the anatomical and chemical development of the cotton embryo in relation to its rate of growth.

The early anatomical development was found to be somewhat irregular, but it showed certain similarities to the early embryonic development of *Malva rotundifolia* as reported by Souèges.

Details of the development of the resin glands were studied. Indications of gossypol were found much earlier in the development of these glands than had been previously reported.

Most of the organs and tissues began their development during the latter part of the formative period and the first part of the grand period of growth. On or before the eighteenth day, oil, starch, pentosans, gossypol, and proteins were formed. All of these materials, except starch, were found throughout the remainder of the growth period.

Glucose was not clearly demonstrated in the embryo at any time during the entire period of development, but it was found in the young lint hairs from the time of their first appearance until just before maturity.

A high degree of variation in rate of growth and in size of embryos within the same boll was found, in spite of the fact that the material was thought to be relatively pure and the flowers had been self-fertilized. Numerous irregularities in form were observed in mature embryos.

LITERATURE CITED

- (1) BALLS, W. L.
1905. THE SEXUALITY OF COTTON. *Khedivia Agr. Soc. Yearbook* 1905: [199]-222, illus.
- (2) FLATTERS, A.
1906. THE COTTON PLANT: ITS DEVELOPMENT AND STRUCTURE AND THE EVOLUTION AND STRUCTURE OF THE COTTON FIBRE. 92 pp., illus. London.
- (3) GALLUP, W. D.
1927. THE GOSSYPOL CONTENT AND CHEMICAL COMPOSITION OF COTTON-SEEDS DURING CERTAIN PERIODS OF DEVELOPMENT. *Jour. Agr. Research* 34: 987-992.
- (4) GORE, U. R.
1932. DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND EMBRYO IN COTTON. *Amer. Jour. Bot.* 19: 795-807, illus.
- (5) MARCHLEWSKI, L.
1899. GOSSYPOL, EIN BESTANDTHEIL DER BAUMWOLLSAMEN. *Jour. Prakt. Chem.* (9N. F.) 60: 84-90.
- (6) SOUÈGES, R.
1922. EMBRYOGÉNIE DES MALVACÉES. DÉVELOPPEMENT DE L'EMBRYON CHEZ LE MALVA ROTUNDIFOLIA L. *Compt. Rend. Acad. Sci. [Paris]* 175: 1435-1436, illus.
- (7) REA, H. E.
1928. LOCATION OF "MOTES" IN THE UPLAND COTTON LOCK. *Jour. Amer. Soc. Agron.* 20: 1064-1068, illus.

THE CHROMOSOME NUMBER IN GLADIOLUS¹

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INTRODUCTION

The genus *Gladiolus* is known best by its many summer-flowering varieties. The exact origin of these types in many cases is uncertain, but it is generally agreed (2, 3, 6, 11, 12, 13)² that interspecific hybridization followed by rigorous selection has played an important part in their development. It appears that such hybrids have often arisen from crosses between very different species and that eventually not only 2, but sometimes 3 and even 4 species have been involved. The high degree of fertility in the commercial varieties, in view of their supposed origin, is surprising.

It was the purpose of this study to determine the chromosome number of many of the species and some of the representative types of the commercial varieties, and, if possible, to establish the relation between the two groups. Subsequent studies will deal with chromosome behavior in interspecific and intervarietal hybrids.

PREVIOUS REPORTS

The literature on chromosome number in *Gladiolus* is not abundant. Most of the reports have given the chromosome number for only a few species or varieties. These are listed in table 1, along with the list of Ernst-Schwarzenbach (5), who has approached this problem along lines similar to those followed in the present investigation.

TABLE 1.—Chromosome numbers previously reported for *Gladiolus*

Species or variety	n	2n	Reported by
<i>G. primulinus</i> Baker:			
var. <i>La Meurthe</i>	30	-----	De Vilmorin and Simonet (14).
var. <i>Priority</i>	14	-----	McLean (10).
<i>G. quartianus</i> A. Rich.....	14	-----	Do.
<i>G. quartianus</i> var. <i>Halloween</i>	14	-----	Do.
<i>G. tristis</i> L.....	14	-----	Do.
<i>G. tristis</i> L. var. <i>concolor</i>	-----	30	Ernst-Schwarzenbach (5).
<i>G. cardinalis</i> Curt.....	-----	30	Do.
<i>G. Colvillei</i> Sweet var. <i>roseus</i>	15	30	Ernst-Schwarzenbach and Britting.
			ham (5).
<i>G. ramosus</i> Paxt.....	-----	46	Ernst-Schwarzenbach (5).
<i>G. cuspidatus</i> Jacq.....	15	-----	Do.
<i>G. byzantinus</i> Mill.....	30	-----	Do.
<i>G. primulinus</i> var. <i>Souvenir</i>	30	-----	Do.
<i>Gladiolus</i> varieties.....	-----	30	Kinoshita (Kihara et al.) (7).
Do.....	-----	60	Wakakuwa (Kihara et al.) (7).
<i>G. gandavensis</i> Van Houtte:			
var. <i>Pompée</i>	30	-----	Ernst-Schwarzenbach (5).
var. <i>Alexandre</i>	-----	60	Do.
var. <i>Red Canna</i>	-----	60	Do.
<i>G. lemoinei</i> Hort.:			
var. <i>Catharina</i>	30	-----	Do.
var. <i>Don Salluste</i>	-----	60	Do.
var. <i>Mrs. Frank Pendleton</i>	-----	60	Do.
<i>G. nanceianus</i> Hort. var. <i>deadmone</i>	30	-----	Do.

¹ Received for publication July 1, 1935; issued February 1936.

² Reference is made by number (italic) to Literature Cited, p. 950.

It appears from table 1 that the larger summer-flowering forms are tetraploid and have a chromosome number of $60=2n$, whereas the smaller winter- or spring-flowering types, both species and varieties, are diploid and have a chromosome number of $30=2n$. One exception is *Gladiolus ramosus* (considered to be a group of hybrids) which has $46=3X+1$ chromosomes and which Ernst-Schwarzenbach (5) considers to be a hypertriploid.

MATERIALS AND METHODS

Seeds and corms were secured from many commercial dealers in South Africa, Europe, and the United States and, whenever possible, duplicate material was used. Some critical material was available through the kindness of Dr. F. T. McLean, of the New York Botanical Garden, and B. Y. Morrison, of the Bureau of Plant Industry, United States Department of Agriculture. Identification was made by resorting to a variety of sources, particularly to the work of Beal (2) and Baker (1). However, the ease of hybridization in *Gladiolus* makes the problem of identification a difficult one, especially where any species has been crossed with a commercial variety. McLean (9) reports that most of the progeny look like the species parent.

The chromosome numbers were determined chiefly from root-tip material, and some were checked in flower buds. Both were fixed with Navaschin's fluid, followed by the usual xylol-paraffin method, and stained in iron-alum haematoxylin after sectioning. Other fixing agents yielded very poor results, particularly when the chromosomes were numerous. Regardless of number, the fixation of the anthers is uncertain, a confirmation of the condition reported by Ernst-Schwarzenbach (5).

All drawings were made with the aid of a camera lucida and the use of a $15\times$ compensating ocular with a 90×1.3 apochromatic objective.

RESULTS AND DISCUSSION

The chromosome number found in species, species hybrids, and various commercial varieties of *Gladiolus* is shown in table 2, and illustrations of the chromosomes of some representative types are presented in figure 1.

It is apparent from table 2 that the genus *Gladiolus* is heteroploid and has a basic chromosome number of 15. The majority of the species are diploid, the only exceptions being the members of the subsection *Dracocephali* and the European-Asiatic group. The former contains the major portion of the species involved in the supposed origin of the summer-flowering commercial varieties. The latter group has always been considered a very distinct one, and this is further indicated by the chromosome number.

The size of the chromosomes, regardless of the number, is almost the same. In fact, any variation might well be covered in one root tip. It is possible at times to detect 2 or 4 large chromosomes but the results are not consistent. Brittingham (3) has also called attention to this fact, and it seems probable that it may account for the counts of 31 found in occasional cells by Ernst-Schwarzenbach (5).

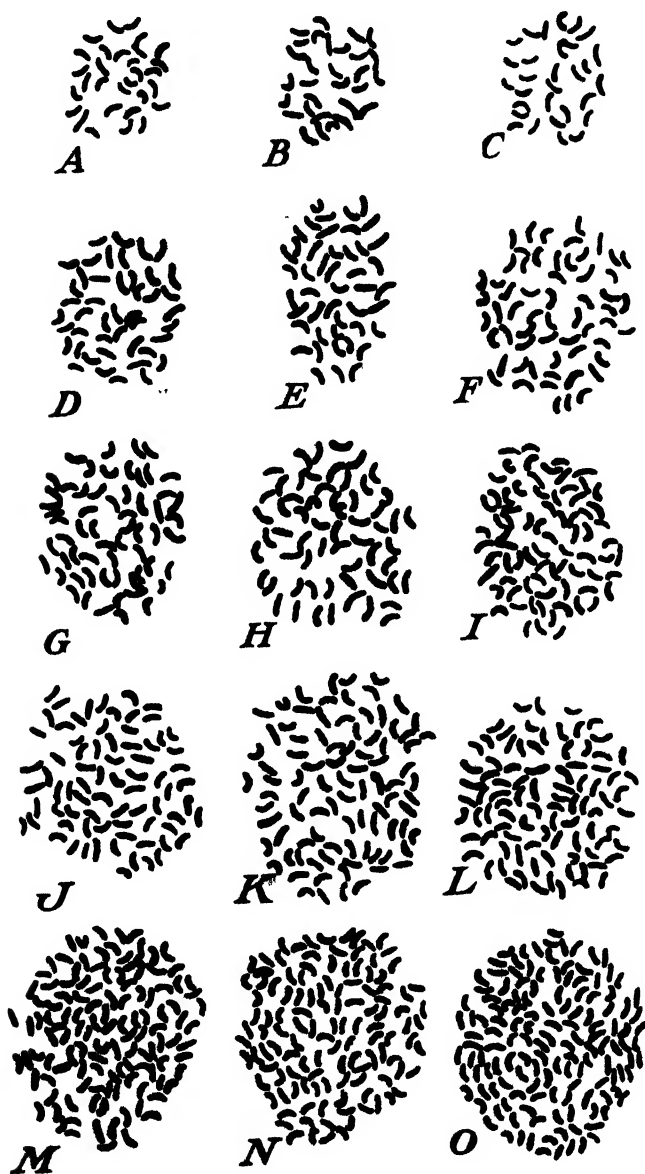


FIGURE 1.—Somatic chromosome plates from root-tip cells of *Gladiolus*: A, *G. cardinalis*, $2n=30$; B, *G. oppositiflorus*, $2n=30$; C, *G. saundersii*, $2n=30$; D, *G. saundersii* hybrid, $2n=45$; E, *Gladiolus* var. Nymph, $2n=45$; F, *G. primulinus*, $2n=60$; G, *G. platyphyllus*, $2n=60$; H, *Gladiolus* var. Mr. W. H. Phipps, $2n=60$; I, *G. dracocephalus* hybrid, $2n=75$; J, *G. quartianus* hybrid, $2n=75$; K, *G. psittacinus*, $2n=90$; L, *G. byzantinus*, $2n=90$; M, *G. segetum*, $2n=120$; N, *G. anatolicus*, $2n=120$; O, *G. communis*, $2n=138\pm$, $\times 2,150$.

TABLE 2.—Chromosome numbers determined for gladiolus species, species hybrids and various commercial varieties

SPECIES AND SPECIES HYBRIDS

Name	n	2n	Name	n	2n
Eugladiolus:			Eugladiolus—Continued		
Species of Europe and western Asia:			Blandi:		
<i>G. byzantinus</i> Mill.	90		<i>G. blandus</i> Aiton.		30
<i>G. communis</i> L. variety.	138±		<i>G. hirsutus</i> Jacq.		30
<i>G. segetum</i> Ker.	120		<i>G. oppositiflorus</i> Herb.		30
<i>G. atroviolaceus</i> Boiss.	45	90	<i>G. undulatus</i> Jacq.	15	30
<i>G. anatolicus</i> Van Tub.		120	<i>G. odoratus</i> L. Bolus.		30
<i>G.</i>		60	<i>G. callistus</i> F. Bolus.		30
Species of Cape and tropical Africa:			Cardinales:		
<i>G. tristis</i> L.	15	30	<i>G. cardinalis</i> Curt.		30
<i>G. tristis</i> var. <i>concolor</i> Salis.	15	30	<i>G. splendens</i> Baker.		30
<i>G. grandis</i> Thunb.		30	<i>G. carmineus</i> Wright.		30
<i>G. recurvus</i> L.		30	Dracocephali:		
<i>G. recurvus</i> hybrid.		30	<i>G. dracocephalus</i> Hook.		90
<i>G. gracilis</i> Jacq.		30	<i>G. dracocephalus</i> hybrid.		75
<i>G. angustus</i> L.		30	<i>G. psittacinus</i> Hook.	45	90
<i>G. cuspidatus</i> Jacq.	15	30	<i>G. psittacinus</i> hybrid.		75
<i>G. trichonemifolius</i> Ker.		30	<i>G. primulinus</i> Baker.	30	60
<i>G. brenifolius</i> Jacq.		30	<i>G. platyphyllus</i> Baker.		60
<i>G. debilis</i> Ker.		30	<i>G. coccineus</i> L. Bolus.		60
<i>G. pappet</i> Baker.		30	<i>G. quartinianus</i> A. Rich. hybrid.		75
<i>G. villosus</i> Ker.		30	<i>G. saundersii</i> Hook.		30
Parviflori:			<i>G. saundersii</i> hybrid.		45
<i>G. crassifolius</i> Baker.		30	Hebea:		
<i>G. papilio</i> Baker hybrid.		75	<i>G. alatus</i> L.	15	30
			<i>G. alatus</i> hybrid.		45
			<i>G. orchidiflorus</i> Andr. hybrid.		45
			<i>G. formosus</i> Klatt hybrid.		45
			<i>G. permeabilis</i> De la Roche.		30

COMMERCIAL VARIETIES, WINTER-FLOWERING TYPES

Name	2n	Name	2n
<i>Gladiolus colvillei</i> Hort.:		<i>Gladiolus nanus</i> Hort.—Continued.	
var. <i>alba</i>	30	var. Robinhood.	30
var. <i>roseus</i>	30	var. Blushing Bride.	30
var. <i>rubra</i>	30	var. Nymph.	45
<i>G. tuberginii</i> Hort.:		var. Liberty.	45
var. Charm.	45	var. Groenendaal.	60
var. Prunella.	45	var. Roos van Dekama.	60
<i>G. nanus</i> Hort.:		Herald gladiolus:	
var. Siren.	30	var. Dillenbergh.	60
var. Splitfire.	30	var. Joost v. d. Vondel.	60
var. <i>cardinalis elegans</i>	30	var. P. C. Hooft.	60
var. Ackermann.	30	var. Prof. Donders.	60
var. Peach Blossom.	30	var. Leeuwenboek.	45

COMMERCIAL VARIETIES, SUMMER-FLOWERING TYPES, (2n=60)

Name	Name	Name	Name
Abbé Raucourt	Commodore	King of Oranges	Pacha
Aida	Contemplation	L von Beethoven	Pfitzer's Triumph
Albatross	Desdemone	Los Angeles	Picardy
Alice Tiplady	Dr. F. E. Bennett	Marshal Foch	Plerian
Alsace Lorraine	Enclade	Mary Jane	Porthos
Altar	Enchantress	Meadow Lark	Pride of Wanakah
Anthony Kunderd	Evelyn Kirtland	Mignot	Princesps
Ave Maria	Francis King	Mr. Mark	Prof. E. H. Wilson
Baron Joseph Hulot	Giant Nymph	Mr. W. H. Phipps	Purple Glory
Blue Isle	Gloriana	Mrs. F. C. Peters	Rob Roy
Blue Triumphator	Golden Dream	Mrs. Frank Pendleton	Rose
Break o' Day	Golden Measure	Mrs. Leon Douglas	Syncopeation
Cardinal Prince	Hyperion	Mrs. P. W. Slson	Taurus
Catharina	Impressario	Mrs. Van Konynen-	Vermillion
Catherine Coleman	Indian Chief	burg	
Cattleya Rose	Jane Addams	October	

While many of the records concerning the development of the commercial varieties of *Gladiolus* may be questionable, there seems to be no doubt that many of the external characters which distinguish some of the species are found in the current commercial types. McLean (10) and others, have shown specific cases and listed the probable species concerned. While the list in table 2 does not include all of these species, it does contain many of the key types, namely, *G. cardinalis*, *G. oppositiflorus*, *G. Saundersii*, *G. primulinus*, *G. dracocephalus*, and *G. psittacinus* and their chromosome numbers are given as 30, 60, and 90. If such species, with different chromosome numbers, are parents of the commercial varieties, it is hard to see how all of these hybrids are tetraploids. However, the occasional triploids and pentaploids might form an intermediate step in this development.

Within the past few years the attention of cytologists has been centered on the nature of the chromosomes rather than on the number, and special consideration has been given to the spindle-fiber attachment, to the satellites, and to size differences. These features have been helpful in tracing the ancestry of existing types. This method of tracing ancestry would be exceedingly difficult to apply in the case of *Gladiolus* because of the smallness of the chromosomes, so other methods must be employed. One of these seems to be a careful observation of the behavior of the triploids and pentaploids, in regard to both chromosome number and genetical characters, when they are used in crosses. That such triploids and pentaploids exist has already been pointed out. These are probably diploid-tetraploid and tetraploid-hexaploid hybrids, and recent crosses between forms with known chromosome numbers have shown that such crosses are very readily secured. If such triploids and pentaploids are again used in backcrosses, particularly as the pollen parent, selection by hybridizers from the resulting offspring might tend to be centered around those with a tetraploid number, because of certain desirable qualities which they possess. This would be especially true when a tetraploid is used as the seed parent. That the gametes of triploids and pentaploids which must effect fertilization are likely to be euploid has been shown in *Zea-Euchlaena*, *Triticum*, and *Nicotiana* hybrids and in many other crosses of this type. Longley, who has listed and discussed these hybrids (8, p. 802) says:

Where the chromosome complement of a plant is made up of chromosomes in addition to the two homologous sets [triploids and pentaploids], the tendency of the functioning gametes to have the basic chromosome number or a multiple of this number must lead to the production of plants with chromosome numbers in multiples of the basic number and to the absence of plants with aneuploid chromosome numbers.

No aneuploid *Gladiolus* has yet been found except a variety of *G. communis*, and this group has apparently not entered into the formation of tetraploid summer gladiolus.

A detailed description of meiosis, particularly in the commercial varieties, is omitted from this account because of the confusing trivalents and tetravalents which need further study.

SUMMARY

The basic chromosome number of the genus *Gladiolus* is 15.

Diploid, triploid, tetraploid, pentaploid, hexaploid, octoploid, and hyperenneaploid species and hybrids have been found. The majority of species are diploid and all of the summer-flowering commercial varieties which were studied are tetraploid.

The subsection *Dracocephali* and the European-Asiatic group contain most of the polyploids.

A brief discussion of the possible origin of the tetraploids, in view of their parentage, is presented.

The chromosomes are small and of approximately the same size.

LITERATURE CITED

- (1) BAKER, J. G.
1892. HANDBOOK OF THE IRIDEAE. 247 pp. London.
- (2) BEAL, A. C.
1916. GLADIOLUS STUDIES—I. BOTANY, HISTORY, AND EVOLUTION OF THE GLADIOLUS. N. Y. Agr. Col. (Cornell) Ext. Bull. 9, pp. 93-188, illus.
- (3) BRITTINGHAM, W. H.
1934. CYTOLOGICAL STUDIES ON SOME GENERA OF THE IRIDACEAE. Amer. Jour. Bot. 21: 77-83, illus.
- (4) CRAWFORD, M.
1921. THE GLADIOLUS; A PRACTICAL TREATISE ON THE CULTURE OF THE GLADIOLUS, WITH NOTES ON ITS HISTORY, STORAGE, DISEASES, ETC. . . . With an appendix by W. Van Fleet. 100 pp., illus. Chicago and New York.
- (5) ERNST-SCHWARZENBACH, M.
1931. CONTRIBUTION A L'ÉTUDE DES CHROMOSOMES CHEZ LE GENRE GLADIOLUS L. Ann. Sci. Nat., Bot. (10) 13: [345]-351, illus.
- (6) HOTTES, A. C.
1915. GARDEN GLADIOLI . . . Jour. Heredity 6: 499-504, illus.
- (7) KIHARA, H., YAMAMOTO, Y., and HOSONO, S.
1931. A LIST OF CHROMOSOME-NUMBERS OF PLANTS CULTIVATED IN JAPAN. pp. 195-330. Tokyo.
- (8) LONGLEY, A. E.
1934. CHROMOSOMES IN HYBRIDS BETWEEN EUCHLAENA PERENNIS AND ZEA MAYS. Jour. Agr. Research 48: 789-806, illus.
- (9) McLEAN, F. T.
1925. THE VALUE OF GLADIOLUS SPECIES FOR HYBRIDIZING. Gladiolus Rev. 2 (5): 7-9, illus.
- (10) ———
1931. INHERITANCE AND CHROMOSOME NUMBER IN THE GLADIOLUS. Torrey 31: 65-70.
- (11) ——— Clark, W. E., and Fischer, E. N.
1927. THE GLADIOLUS BOOK. 233 pp., illus. Garden City, N. Y.
- (12) PRIDHAM, A. M. S.
1932. THE GLADIOLUS: ITS HISTORY, CLASSIFICATION, AND CULTURE. N. Y. Agr. Col. (Cornell) Ext. Bull. 231, 65 pp., illus.
- (13) SANDHACK, H. A.
1927. DAHLIEN UND GLADIOLEEN, IHRE BESCHREIBUNG, KULTUR UND ZÜCHTUNG; EIN HANDBUCH FÜR DIE PRAXIS DES BERUFSGÄRTNERS UND GARTENLIEBHABERS. 268 pp., illus. Berlin.
- (14) VILMORIN, R. DE, and SIMONET, M.
1927. NOMBRE DES CHROMOSOMES DANS LES GENRES LOBELIA, LINUM ET CHEZ QUELQUES AUTRES ESPÈCES VÉGÉTALES. Compt. Rend. Soc. Biol. 96: 166-168, illus.

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EVIDENCE OF VIRUS MUTATION IN THE COMMON MOSAIC OF TOBACCO¹

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INTRODUCTION

In a short note (12)² the writer reported that Connecticut-Havana broadleaf tobacco³ plants having common mosaic developed yellow spots on a few leaves, as previously illustrated (15), and as shown in figure 1, B. These yellow spots were observed regularly on plants in the more advanced stages of development, and very irregularly on plants when young, and then only when the young plants were growing at high temperatures.⁴

These yellow spots range in size from points just visible to areas approximating 1.5 cm² on the strain of tobacco used. On Turkish tobacco the spots are smaller and have been less consistent in occurrence. The large spots are less frequent, usually are very irregular in shape and not so yellow in color as the small spots, though small spots are not always completely devoid of chlorophyll. On this account one cannot be certain from mere observation that mosaic tissue is free of very small invisible centers of yellow-mosaic virus. Figure 2 illustrates a typical series of sizes and shapes of the yellow-mosaic spots.

It was found that these yellow spots contain virus which when introduced into young tobacco plants induced a large number of yellow areas on the young leaves, usually within 4 to 5 days at temperatures favorable for tobacco culture. A typical case is illustrated in figure 3, A. It was found that a very severe pure yellow mosaic sometimes could be obtained immediately in the inoculated plants when the virus was taken from the center of extremely yellow areas. When virus was taken from near the margins of the yellow areas, blends of yellow mosaic and green mosaic developed on many of the inoculated plants, and when virus was taken from the green areas common mosaic developed in the inoculated plants, but all of these plants ultimately developed a few small spots of yellow mosaic. The virus of common mosaic was completely removed from the yellow mosaic by successive subinoculations from the yellow-mosaic areas. Several leaves with yellow mosaic are illustrated in figure 1.

¹ Received for publication Aug. 26, 1935; issued February, 1936. These studies were started in 1926 at the University of Wisconsin and continued at the Arlington Experiment Farm, Rosslyn, Va.

² Reference is made by number (italic) to Literature Cited, p. 980.

³ This variety, which was used throughout the present experiments, is now called Wisconsin-Havana Seed.

⁴ It was stated (12) inadvertently that these yellow spots developed on young plants grown near 90° to 100° C. This temperature reading was taken from a Fahrenheit thermograph chart and through oversight was not converted to the centigrade scale. It should be read 32.2° to 37.7° C. These were midday temperatures during midsummer.

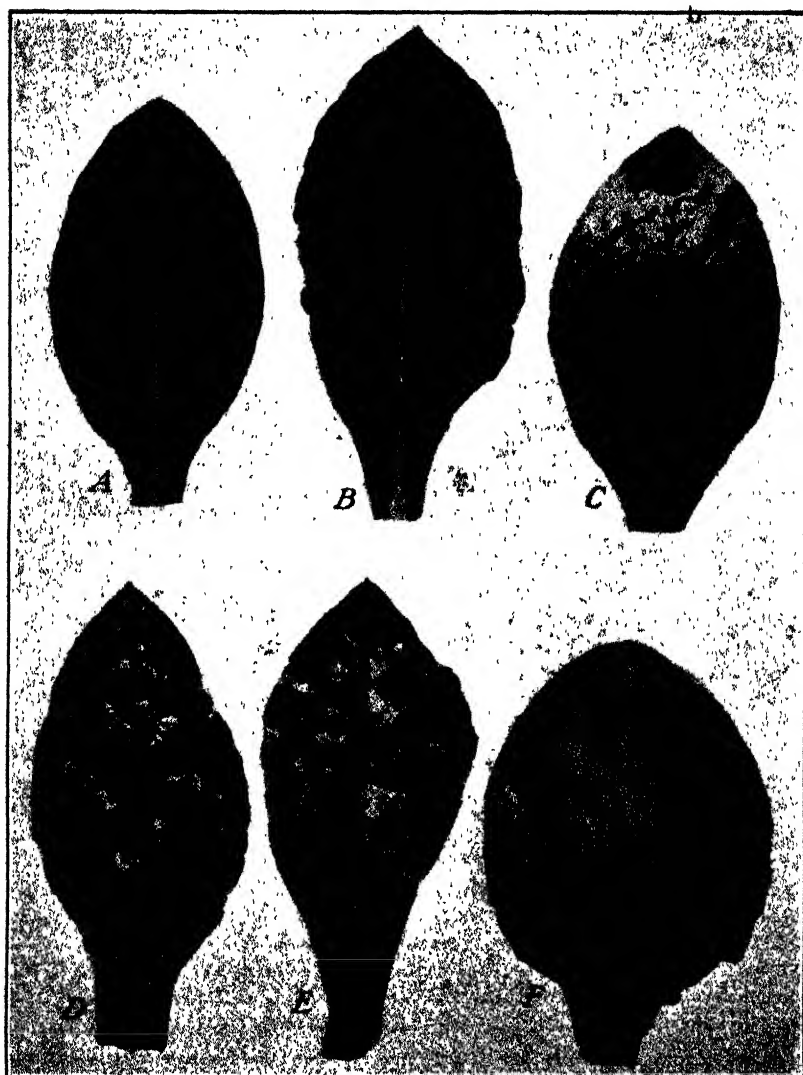


FIGURE 1.—Common mosaic and yellow mosaic on tobacco. *A*, Healthy leaf; *B*, leaf showing common mosaic and one yellow-mosaic spot; *C*, *D*, *E*, and *F*, several patterns of yellow mosaic produced by purified virus obtained from a yellow-mosaic spot such as is illustrated in *B*.

Many collections of mosaic have been studied, and as stated previously (13, 15, 19) all those having the appearance of common mosaic on tobacco have shown the presence of yellow-mosaic spots on one or more leaves on all of the inoculated plants. All attempts to obtain a common mosaic which does not develop yellow-mosaic spots failed.

In view of these facts it was pointed out (15) that the virus of common mosaic may mutate. In a later summary (19) the writer took the definite position that common green mosaic and the yellow mosaic associated with it do not represent an ordinary mixture of viruses. No statement was made at that time regarding mutation. However, common mosaic was referred to as a complex because all the evidence indicated that this disease on Wisconsin-Havana Seed tobacco when cultured under ordinary conditions represents a composite or aggregate of at least two types of mosaic—green mosaic which predominates and yellow mosaic which occurs in localized zones—and because of the never-failing recurrence of the yellow-

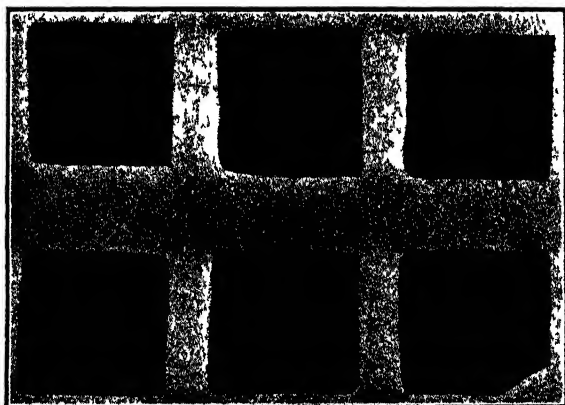


FIGURE 2.—Types of yellow-mosaic spots ranging from a small point to a large type in which some chlorophyll is present. Natural size.

mosaic phase with the green-mosaic phase of the disease in the writer's tests.⁵

Dufrenoy (4), working in France, and Jensen (8), in the United States, have verified the writer's findings that yellow mosaic is associated with the common mosaic of tobacco. Price (23) and Hoggan (5) have reported a similar phenomenon in cucumber mosaic. The writer (16, 18) has pointed out that certain yellow and green mosaics of wheat are closely associated. Jensen (8) and Price (23) and Hoggan (5) consider that these associations in tobacco and cucumber mosaics do not constitute mixtures of an ordinary type, and that the viruses of yellow-mosaic spots arise in the plant sometime after inoculation.

The present paper deals with several lines of evidence which support the view that the viruses which cause the yellow-mosaic spots arise spontaneously as mutations of the virus of common mosaic, and are not the result of mixture or contamination of the ordinary

⁵ Smith (26) reports a virus disease relationship in potato which he refers to as a complex, but this seems to represent an ordinary mechanical mixture of viruses. Thus, it is seen that the writer used the term complex in a different sense.



FIGURE 3.—*A*, first symptoms induced by virus from a yellow-mosaic spot; *B*, symptoms of common mosaic showing no yellow-mosaic spots induced by virus from the light- and dark-green areas of common mosaic. The new leaves shown in *A* and the subsequent leaves produced symptoms of common mosaic which were indistinguishable from the symptoms produced by plant *B*. Both plants produced a few yellow-mosaic spots before maturity.

sort running through all collections of common mosaic and through all of the many lots and fractions of inoculum used in the experimental work.

MATERIALS, METHODS, AND TERMINOLOGY

The studies on tobacco were confined to a strain of Wisconsin-Havana Seed (Havana No. 38) unless stated otherwise. The seed was obtained from successive cuttings from a single plant, and precautions were taken to prevent cross-pollination. Tests were carried out from time to time with Turkish tobacco, and while this type had certain advantages, these were offset by disadvantages not possessed by the strain of tobacco used. The studies on *Nicotiana glauca* R. Grah. were confined to a strain collected by the writer on the island of Tenerife in the Canary group. *N. rustica* L., *N. glutinosa* L., *N. sylvestris* Spegaz. and Comes, and a strain of *N. affinis* Hort. were employed in some of the tests. Seed and young plants of these species were supplied by E. E. Clayton and J. E. McMurtrey.

Unless otherwise stated, the common-mosaic virus used originated from material supplied the writer by James Johnson, the eleventh source recorded in table 1. Previous to the present studies, dilution tests were carried out to determine the power of increase of the virus in tobacco. A series of dilutions was made up to 100,000 in water. Each of these was inoculated into five young tobacco plants. Soon after the appearance of the common-mosaic mottling, virus extract was obtained from one plant in the highest dilution series in which symptoms were expressed. This extract was again diluted in series and inoculated into tobacco plants. This procedure was continued through seven dilution tests. Virus was taken from plants inoculated with extract diluted 10,000 times in 3 tests, and from plants inoculated with extract diluted 100,000 times in 4 tests. It was in the eighth test that all plants with mosaic were allowed to grow to maturity, and it was in these plants that the yellow-mosaic spots were first noted.

During these early tests only the common mosaic was in the greenhouse, and in the first seven tests no yellow-mosaic spots appeared on the young plants before they were discarded. The virus of common mosaic used in all the detailed studies originated from the plants in the eighth dilution test and from leaves which were free of yellow-mosaic spots. Throughout all the work this virus has been guarded to prevent contamination from outside sources, and transfers have been made from tissue which appeared to be free from yellow-mosaic spots, though it is recognized that exceedingly small zones of yellow-mosaic virus not detectable are very likely to be present in some of these tissues.

The mild dark-green mosaic ⁶ (15) used in the tests was isolated from a mixture of mosaics, one of which is a light-green mosaic (common mosaic) which in turn developed yellow-mosaic spots. The original mixture came from mosaic *Nicotiana glauca* plants collected by the writer in the Canary Islands. The mild dark-green mosaic did not develop yellow-mosaic spots in the tests reported in this paper.

⁶ Pigment analyses carried out by Peterson (21) show that chlorophyll reduction is less in tobacco plants with mild dark-green mosaic than in plants with common mosaic. This relationship is indicated also in the appearance of the plants after mild dark-green mosaic has passed through the mild stage referred to in an earlier paper (16). Common mosaic is comparatively light green in color and was referred to as light-green mosaic in another paper (15).

However, if plants with this mosaic become reinfected with common mosaic, yellow-mosaic spots may appear before the presence of common mosaic becomes evident. Isolations from such spots have always shown the presence of the common-mosaic virus as well as a yellow-mosaic virus.

Purified yellow mosaics, type A and type B, briefly described in this paper, were also used. The type A yellow mosaic was used in most of the tests as it was isolated from the common mosaic also used throughout the tests. Viruses of these yellow mosaics are referred to as "pure" because present methods fail to demonstrate the presence of other viruses. In perpetuating these mosaics the virus extract was always taken from yellow areas on the leaves, but when large quantities of extract were required entire leaves were used. Several other collections of virus were used in the survey tests, but these were not employed in the detailed studies.

The routine inoculations were made by the needle-cotton method previously described (14), unless otherwise stated.

All plants with common mosaic which were used for the yellow-mosaic spot observations were grown to maturity unless the spots appeared earlier. In a few cases plants were cut back when yellow-mosaic spots did not appear before maturity in order to give an opportunity for yellow-mosaic spots to develop on new leaves. This cutting was done with pruning shears; these and the hands of the operator were thoroughly washed in 95-percent alcohol before the pruning of each plant.

Ample uninoculated controls were employed. Pots were sterilized with steam before use. Fertile soil was used and a 1-percent calcium nitrate solution was applied to the plants at intervals during the tests. Soil was checked for the presence of virus by close observation of the healthy plants. The greenhouses used at Madison, Wis., were not screened against insects, but those at the Arlington farm were screened with 30-mesh copper-wire gauze. Fumigations were made at intervals as a safety measure. Plants inoculated with a given virus extract were spaced to prevent their touching plants inoculated with another virus extract, and in certain studies screen partitions were used as previously illustrated (15, *fig. 6*). Pots were set on low benches or on sterilized boards on the ground.

During the period of the Allison V. Armour expedition of collection (1926-27) (15) many inoculation tests were carried out with tobacco plants grown in pots on the upper deck of the yacht, while on the Atlantic Ocean. In these tests the vector problem was reduced to a minimum.

The routine temperatures of the greenhouses were near 21° to 24° C. in winter unless otherwise stated. During bright days the temperature sometimes went higher. In the summer the temperatures were slightly above those outdoors. The roof and sides of the greenhouses were whitewashed during the spring and summer months.

In this paper the term "mutation" is used to designate the "breaking-up" characteristic of the virus of common mosaic because the evidence to be presented indicates that the new viruses are not contaminants from outside sources and because they represent permanent departures from an established type.

The terms "type" and "strain" are used more or less interchangeably to designate viruses differing from each other irrespective of the

degree of difference. This usage seems to be permissible until there is a more complete agreement among investigators on points relating to the relative importance of virus characteristics and to the usage of terms.

INOCULATION SURVEY OF COLLECTIONS

Mosaic viruses were obtained from 26 sources and tested on Havana Seed tobacco. As indicated in table 1, 23 of these were common mosaic or mosaics resembling common mosaic, and 3 were distinctly different from common mosaic. All plants were closely observed for the development of yellow-mosaic spots, and it was found that, without exception, all plants manifesting common mosaic or a mosaic resembling common mosaic developed one or more yellow-mosaic spots which were either bright yellow or yellow intermingled with green.

It was not possible to make isolations from the yellow-mosaic spots appearing on each plant, but isolations were made from time to time, and without fail, when the spots were large enough to yield sufficient virus, yellow mosaics eventually were established. In the case of very small spots frequently there was insufficient virus to induce yellow mosaic in the inoculated plants.

TABLE 1.—*Inoculation tests with mosaic viruses obtained from different localities and laboratories to determine the occurrence of yellow-mosaic spots*

Type of mosaic on tobacco	Locality where obtained	Collaborator or collector	Plant on which collected	Tobacco plants on which mosaic symptoms developed	Plants which developed yellow-mosaic spots	Places where tests were conducted ¹
				Number	Number	
Light-green mosaic (common mosaic).	Connecticut.....	G. P. Clinton...	Tobacco.....	10	10	A, B
Do.....	Florida.....	W. B. Tisdale.....	do.....	5	5	B
Do.....	Louisiana.....	H. H. McKinney.....	Tomato.....	15	15	A, B
Do.....	do.....	do.....	do.....	5	5	B
Do.....	Missouri.....	B. M. Duggar.....	Tobacco.....	10	10	A, B
Do.....	New York.....	F. M. Blodgett.....	do.....	5	5	A
Do.....	Virginia.....	H. A. Allard.....	do.....	3	3	B
Do.....	do.....	do.....	do.....	3	3	B
Do.....	do.....	Walter Marcey.....	Garden pepper.....	5	5	B
Do.....	do.....	S. A. Wingard.....	Tobacco.....	15	15	A, B, C
Do.....	Wisconsin.....	James Johnson.....	do.....	4, 224	4, 224	A, B, C
Do.....	do.....	S. P. Doolittle.....	do.....	5	5	B
Do.....	Hawaii.....	J. Atherton Lee.....	do.....	15	15	A, B
Do.....	England.....	A. J. Riker.....	do.....	10	10	B
Do.....	Island of Tenerife.....	H. H. McKinney.....	<i>Nicotiana glauca</i>	15	15	B, C
Do.....	Island of Grand Canary.....	do.....	do.....	10	10	B
Resembles common mosaic.	do.....	do.....	do.....	5	5	B
Do.....	do.....	do.....	do.....	5	5	B
Do.....	do.....	do.....	do.....	10	10	B
Do.....	do.....	do.....	do.....	5	5	B
Do.....	do.....	do.....	do.....	5	5	B
Do.....	do.....	do.....	do.....	10	10	B
Do.....	do.....	do.....	do.....	5	5	B
Mild dark-green mosaic.	do.....	do.....	do.....	318	10	B
Do.....	Gibraltar.....	do.....	do.....	62	0	B
Mild mosaic.....	Ephrata, Pa.....	do.....	Tobacco.....	371	10	B

¹ The letters A, B, and C indicate that the test was conducted at Madison, Wis.; Roslyn, Va.; or on shipboard, respectively.

² Yellow mosaic dominated 3 plants with mild dark-green mosaic and 2 plants with mild mosaic due to accidental contamination.

Healthy plants remained free of these spots. An occasional case of common mosaic and of concentrated yellow mosaic did develop out of the many thousands of healthy tobacco plants grown for various and sundry purposes, but plants used for healthy controls remained free of visible evidence of mosaic.

While all of these collections have not been given intensive study, observations indicate that all collections of mosaic which ordinarily pass for common mosaic on tobacco are not identical in their expression of symptoms on tobacco or on *Nicotiana glauca*. One of the collections from Virginia differs slightly in the mosaic pattern, and the collection from Hawaii has been characterized by a more pronounced expression of the yellow-mosaic spots than other collections.

Three green mosaics which differ from common mosaic were studied on tobacco regularly in the same greenhouses in which the studies on common mosaic and concentrated yellow mosaic were being conducted. The first mosaic in this group is the mild dark-green type. Many of the properties of the virus of this mosaic are similar to those of common mosaic, but it produces no mottling on tomato, and all tests indicate that the tomato plant does not carry the virus. Mild dark-green mosaic has not developed yellow-mosaic spots in the writer's tests. However, of the 138 cases of this disease which were studied, one leaf on one plant was observed to develop a single small light-green mosaic spot. This spot when isolated and inoculated into healthy plants induced a mosaic resembling the mild dark-green mosaic, but light-green semitranslucent spots developed soon after inoculation and the leaves became very deformed, in contrast to the more nearly normal-shaped leaves on plants having the mild dark-green mosaic. The rarity of the occurrence of the light-green spots on plants with mild dark-green mosaic makes it difficult to interpret the origin of this new virus, but it seems reasonable to believe they may originate in the same manner as the yellow-mosaic spots associated with common mosaic.

The second mosaic of this group resembles the first, but differs somewhat in symptoms. It was collected by the writer on *N. glauca* at Gibraltar (15). It has not shown the presence of yellow-mosaic spots.

The third mosaic of this group develops mild mottling throughout the life of the plant. It was collected by the writer in a commercial tobacco field at Ephrata, Pa. The virus can be maintained for only a very short period outside the plant, and it is usually more severe on tomatoes than common mosaic; it induces mosaic on Ambalema tobacco, the variety which Nolla et al. (20) found to be resistant to common mosaic. This mild mosaic has not developed local spots of yellow mosaic on Wisconsin-Havana Seed tobacco.

These tests suggest that the yellow-mosaic spots are universally associated with common mosaic of tobacco when test conditions are suitable, and they indicate that these spots are not expressed by certain other mosaics, at least under conditions favoring their expression in common mosaic.

ATTEMPTS TO REMOVE PERMANENTLY ALL TRACES OF YELLOW MOSAIC FROM COMMON MOSAIC**TESTS ON TOBACCO WITH VIRUS SUBJECTED TO DILUTIONS AND TO DIFFERENT PHYSICAL AND CHEMICAL TREATMENTS**

Tests have indicated that there is not a great deal of difference between the virus of type A yellow mosaic and the virus of common mosaic with respect to dilution in water. The data in table 2 indicate practically no difference, but in the course of many incidental tests it has been observed that the virus of type A yellow mosaic sometimes fails to induce mosaic in all the inoculated tobacco plants when the virus extract is diluted 1,000 times in water, whereas the virus of common mosaic when diluted 1,000 times has always induced mosaic in all of the inoculated tobacco plants.

When fresh extracts containing these viruses were mixed in equal parts the symptoms on inoculated tobacco plants were a blend between those of common and those of yellow mosaic, the tissues that showed the green or common mosaic being in excess of those that showed the yellow mosaic. Mixtures of inoculum which contained the small amounts of the virus extract of yellow mosaic induced less yellow mosaic than mixtures which contained the larger amounts. It will be noted in table 3 that the extracts which contained the smaller proportions of yellow-mosaic virus did not induce blends in all of the plants in a given series. Most of the plants developed only common mosaic during the first few weeks after inoculation, followed by a few of the yellow-mosaic spots characteristic of common mosaic. Mixtures containing 99 or more parts of type A yellow-mosaic extract to 1 part of common-mosaic extract developed intense yellow mosaic soon after inoculation, and the symptoms were like those of the plants which were inoculated with the pure virus of yellow mosaic. As these plants continued their growth common mosaic gradually dominated the new tissues, as indicated in table 4.

TABLE 2.—Comparative responses of the virus of common mosaic and the virus of type A yellow mosaic to dilution with water, and the numbered leaves showing yellow-mosaic spots on plants with common mosaic, and the approximate size of these spots on each leaf

Plant no.	No dilution				Diluted 1,000 X				Diluted 10,000 X				Diluted 50,000 X				Diluted 100,000 X			
	Common mosaic				Common mosaic				Common mosaic				Common mosaic				Common mosaic			
	Disease present or absent	Leaves on which yellow-mosaic spots appeared ¹	Approximate mean diameter of yellow-mosaic spots ²	Yellow mosaic (disease present or absent)	Disease present or absent	Leaves on which yellow-mosaic spots appeared ¹	Approximate mean diameter of yellow-mosaic spots ²	Yellow mosaic (disease present or absent)	Disease present or absent	Leaves on which yellow-mosaic spots appeared ¹	Approximate mean diameter of yellow-mosaic spots ²	Yellow mosaic (disease present or absent)	Disease present or absent	Leaves on which yellow-mosaic spots appeared ¹	Approximate mean diameter of yellow-mosaic spots ²	Yellow mosaic (disease present or absent)	Disease present or absent	Leaves on which yellow-mosaic spots appeared ¹	Approximate mean diameter of yellow-mosaic spots ²	Yellow mosaic (disease present or absent)
1	+	20, 35	1.2	+	+	16, 20 18, 25 32, 33	9.2 7.1, 2.5 5	+	+	40, 41 18, 31, 32	5.5 4.1, 5	+	0		Mm	0	0			0
2	+	45	2	+	+			+	+			+	0				0			0
3	+	30	5	+	+	19, 31 41	3, 4	+	+			+	0				0			0
4	+	5, 30, 37	1.7, 1.1	+	+	19, 31 42	3, 4	+	+			+	0				0			0
5	+	30, 37	3	+	+	11, 30 27	4.2	+	+	30	4	+	+	20	12		+	10, 40	3.6	+
6	+	41	2	+	+		4, 2	+	+			+	0				0			0
7	+	12	3	+	+	13	1	+	+			+	0				0			0
8	+	21	3.4	+	+	9	3	+	+			+	0				0			0
9	+	14, 20, 21	6.3, 4	+	+	43	5	+	+	15, 19	5.7	+	0			+				0
10	+	43	3	+	+			+	+			+	0				0			0

¹ The numbers in this column indicate which leaves developed yellow-mosaic spots. The number 20, for example, signifies the twentieth leaf. The numbering starts with the first leaf to show common mosaic.

² The figures in this column indicate the approximate area of the yellow-mosaic spots on each leaf on each plant in terms of the approximate mean diameter in millimeters.

TABLE 3.—Types of symptoms produced on tobacco when inoculated with synthetic mixtures of virus extracts of common mosaic and of type A yellow mosaic and with each virus separately

[Inoculations made June 22, 1927; 5 plants inoculated with each extract]

Parts of virus extracts used in the mixtures		Notes taken July 8	Notes taken July 26	Notes taken Aug. 8
Virus extract of common mosaic	Virus extract of yellow mosaic			
1	0	5 plants common mosaic.	5 plants had common mosaic, 1 to 3 yellow-mosaic spots on each plant.	5 plants had common mosaic, with 1 to 4 yellow-mosaic spots on each plant.
999	1	5 plants common mosaic, but 1 plant had yellow-mosaic spots.	New leaves on 5 plants had common mosaic with 1 or 2 yellow-mosaic spots per plant.	New leaves on 5 plants had common mosaic with 1 to 3 yellow-mosaic spots per plant.
499	1	5 plants common mosaic, but 2 plants had yellow-mosaic spots.	New leaves on 5 plants had common mosaic with 1 to 3 yellow-mosaic spots per plant.	New leaves on 5 plants had common mosaic with 1 to 4 yellow-mosaic spots per plant.
99	1	do.....	New leaves on 5 plants had common mosaic with 1 to 4 yellow-mosaic spots on each plant.	Do.
1	1	5 plants common and yellow mosaic, but common mosaic predominated.	5 plants had many yellow-mosaic spots on new leaves, but common mosaic predominated.	New leaves on 5 plants had common mosaic with 1 or 2 yellow-mosaic spots per plant.
1	99	5 plants had symptoms of yellow mosaic only. Looked like yellow-mosaic control.	5 plants had severe yellow mosaic, but more green tissue than yellow-mosaic control.	Do.
1	499	do.....	5 plants looked like the yellow-mosaic controls.	All 5 plants had much yellow mosaic, but common mosaic was showing in top leaves of 5 plants.
1	999	do.....	do.....	All plants had much yellow mosaic, but common mosaic was showing in top leaves of 4 plants.
0	1	5 plants had symptoms of yellow mosaic only.	5 plants had symptoms of pure yellow mosaic.	5 plants had symptoms of pure yellow mosaic in all of the leaves.

TABLE 4.—Progressive change in type of symptoms on tobacco leaves as the plants developed

Observations were made on each plant which was inoculated with the virus mixture containing 1 part of extract of common mosaic and 99 parts of extract of type A yellow mosaic in the experiment cited in table 3. [Notes were taken Aug. 8. Leaf numbers start with the first leaf to show symptoms after inoculation]

Type of symptoms on leaves	Leaf numbers in each symptom group on each plant				
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
Dead; originally all yellow mosaic.....	1-5	1-4	1-5	1-5	1-4
Entirely yellow mosaic.....	6-13	5-16	6-13	6-13	5-11
Blends of common mosaic and yellow mosaic.....	14-20	17-22	14-21	14-19	12-16
Common mosaic with the occasional yellow-mosaic spots.....	21-27	23-26	22-28	20-28	17-27

In an earlier paper (15) it was pointed out that tobacco plants having common mosaic show no changes in symptoms after being reinoculated with the pure undiluted virus of yellow mosaic. The same results have been obtained with all the yellow mosaics which

the writer has isolated from tobacco and tested against common mosaic. Thung (27) later reported similar observations, and he reports that tobacco plants having the yellow or "white" mosaic show no change in symptoms when reinoculated with the virus of common mosaic. The writer's results are not in accord with those of Thung on this point. When plants with type A yellow mosaic growing at temperatures of about 21° to 24° C. were reinoculated with a fresh undiluted virus extract of common mosaic, from 5 to 20 of the subsequent leaves exhibited yellow mosaic, the characteristic green mottling of common mosaic then gradually appeared, and from 8 to 25 leaves developed blended symptoms, the green mottling of common mosaic gradually gaining in proportion to the yellow. The subsequent leaves exhibited the characteristic mottling and the occasional small yellow-mosaic spots typical of common mosaic. Virus from light- and dark-green mottled portions of these leaves induced common mosaic when inoculated into healthy tobacco plants. When the stems of mature plants were cut back leaving only the portions which had developed the yellow-mosaic leaves prior to the reinoculation, it was found that the buds produced branches which developed typical common mosaic. Yellow-mosaic control plants which were not reinoculated exhibited typical yellow mosaic throughout the test, and the lower side shoots developed typical yellow mosaic when the stems were cut back. Thung may have discarded his test plants too soon; also it is possible that his method, his viruses, his tobacco, or the environment may account for his negative results.

From the results presented in the foregoing paragraphs, it seems reasonable to conclude that the virus of yellow mosaic either is not present in available form in tissues showing only the common green mosaic or if it is present the number of virus particles is considerably smaller than the number of particles of common-mosaic virus. Therefore, on diluting a virus extract obtained from leaves showing only the common green mosaic, a point should be reached where the number of particles of the yellow-mosaic virus would be nil or too small to produce infection, but where there would be a sufficient number of particles of the other virus to induce infection. On the basis of random assortment, a high percentage or all of the infected plants resulting from such a dilute virus should develop the common green mosaic free from yellow-mosaic spots.

Dilution tests were carried out and the plants were held for close observation throughout the entire life cycle. Plants which developed common mosaic also developed yellow-mosaic spots, and there was no essential difference in the relative time of their appearance or in the range in number or size of the spots produced on plants inoculated with dilute virus as compared with plants inoculated with concentrated virus. The results of a typical test are shown in table 2. These results were unexpected, for it was thought that the virus of yellow mosaic could be readily eliminated from the virus of common mosaic. The phenomenon appeared to represent some unusual type of mixture or a mutation of the common-mosaic virus. With these possibilities in mind further tests were carried out.

It was found that dilute virus of common mosaic was inactivated at slightly lower temperatures than concentrated virus (13, 22). Accordingly extracts were diluted 1,000 times in water and treated for 10 minutes at temperatures ranging from 85° to 90° C. All the

inoculated plants that developed symptoms, first expressed the green mottling typical of common mosaic, which was indistinguishable from the symptoms in plants inoculated with the undiluted unheated virus. As the plants developed, the typical yellow-mosaic spots appeared on from 1 to 6 leaves on each plant, and the symptoms in the plants inoculated with the heated virus were essentially indistinguishable from those in the plants inoculated with the unheated virus.

In view of the previous results it seemed strange that these treatments failed to eliminate the yellow-mosaic virus if it represented a mixture. Accordingly, especial attention was given to methods for preventing the possible accidental contamination of the experimental materials.

As the studies progressed and it was found that the purified mild dark-green mosaic collected in the Canary Islands failed to manifest yellow-mosaic spots, it became evident that this mosaic might prove of value in testing for the separation of yellow-mosaic virus from it in synthetic mixtures. When these viruses were blended in different proportions the plants inoculated with mixtures containing large proportions of virus of yellow mosaic developed only the symptoms typical of pure yellow mosaic. When the proportions of virus of mild dark-green mosaic were increased some of the inoculated plants developed only yellow mosaic, some expressed blended symptoms followed by intense yellow mosaic, and some showed symptoms of mild dark-green mosaic with no signs of yellow mosaic. The results of two tests with mixtures including type A yellow-mosaic virus are presented in table 5.

TABLE 5.—Types of symptoms expressed by tobacco plants inoculated with synthetic mixtures of virus extracts of mild dark-green mosaic and type A yellow mosaic, and with each virus separately¹

TEST 1

Plant no.	GM only	1 part GM, 1 part YM	9 parts GM, 1 part YM	49 parts GM, 1 part YM	99 parts GM, 1 part YM	249 parts GM, 1 part YM	YM only
1.....	G	Y	Y	GY	G	G	Y
2.....	G	Y	Y	GY	GY	G	Y
3.....	G	Y	Y	GY	GY	G	Y
4.....	G	Y	Y	GY	GY	G	Y
5.....	G	Y	Y	GY	GY	Y	Y

TEST 2

Plant no.	GM only	1 part GM, 1 part YM	9 parts GM, 1 part YM	99 parts GM, 1 part YM	499 parts GM, 1 part YM	999 parts GM, 1 part YM	YM only
1.....	G	Y	Y	Y	GY	Y	Y
2.....	G	Y	Y	GY	GY	GY	Y
3.....	G	Y	GY	GY	GY	GY	Y
4.....	G	Y	GY	GY	GY	GY	Y
5.....	G	Y	GY	GY	GY	G	Y

¹ GM and YM in the box heads indicate virus extract from mild dark-green mosaic and from yellow mosaic, respectively. G and Y in the columns indicate the symptoms of mild dark-green and of yellow mosaic, respectively, in the plants that were inoculated with the virus mixtures or with pure viruses as indicated. In the case of all plants that developed the symptoms of both mosaics, the symptoms of green mosaic eventually were lost and only the yellow mosaic was expressed by the new leaves.

Healthy tobacco plants and tomato plants were inoculated with virus obtained from plant no. 5 in table 5, test 2, which had been inoculated with 999 parts of extract from mild dark-green mosaic to 1 part of extract from yellow mosaic. Tomatoes are not susceptible to this green mosaic, but are very susceptible to type A yellow mosaic. The tomato plants remained free of mosaic and the tobacco plants developed mild dark-green mosaic with no signs of yellow-mosaic spots.

It was pointed out in another paper (15) that plants with mild dark-green mosaic when reinoculated with the virus of yellow mosaic ultimately developed yellow mosaic on the new foliage, the reverse of the situation in common mosaic. Blended symptoms developed first, followed later by yellow mosaic which crowded out the mild dark-green mosaic. Plants showing the blended stage in the symptoms frequently produced leaves with rather large areas which were devoid of yellow-mosaic spots. Such areas were used to supply virus for inoculating healthy tobacco plants. In one test five healthy plants were inoculated with undiluted extract from such tissue and all plants developed yellow mosaic. The same extract was diluted to 1,000 times its original volume in sterile distilled water and used to inoculate five healthy tobacco plants. Each plant developed mild dark-green mosaic and each was free from yellow-mosaic spots throughout the life cycle.

When the virus of type B yellow mosaic was mixed with the virus of mild dark-green mosaic in the proportion of 1 part to 99 parts, only 1 plant of the 10 inoculated developed mild dark-green mosaic and showed no trace of yellow mosaic. The results of this test are presented in table 6.

TABLE 6.—*Expression of green mosaic, yellow mosaic, or blends of the two, by tobacco plants inoculated with synthetic mixtures of virus extracts of mild dark-green mosaic and type B yellow mosaic and with each virus separately*¹

Plant no.	GM only	99 parts GM, 1 part YM	249 parts GM, 1 part YM	YM only	Plant no.	GM only	99 parts GM, 1 part YM	249 parts GM, 1 part YM	YM only
1.....	G	GY	GY	Y	6.....	-----	GY	GY	-----
2.....	G	GY	GY	Y	7.....	-----	GY	GY	-----
3.....	G	GY	GY	Y	8.....	-----	GY	GY	-----
4.....	G	GY	GY	Y	9.....	-----	GY	GY	-----
5.....	G	GY	GY	Y	10.....	-----	G	GY	-----

¹ GM and YM in the box heads indicate virus extract from mild dark-green mosaic and from yellow mosaic, respectively, in the inoculum. G and Y in the columns indicate the symptoms of mild dark-green and of yellow mosaic, respectively, in the plants that were inoculated with the virus mixtures or with pure viruses as indicated. In the case of all plants that developed the symptoms of both mosaics, the symptoms of green mosaic eventually were lost and only the yellow mosaic was expressed by the new leaves.

The results obtained with the mixtures of mild dark-green mosaic and the yellow mosaics are in accord with the results expected from ordinary mixtures or contaminations in which an independent random distribution of the units or particles occurs. The fact that yellow mosaics could be eliminated from the mild dark-green mosaic without resorting to extremely high dilutions or special treatments, but could not be eliminated from the diluted virus of common mosaic, is a strong indication that the virus of the yellow-mosaic spots does not constitute an ordinary contamination from an outside source, but arises in the plant after the onset of common mosaic.

While these tests strongly suggested that the virus of yellow mosaic represented a mutant, it seemed advisable to give consideration to the hypothesis that this virus has an inactive stage caused by adsorption or some other phenomenon, and that the yellow-mosaic spots develop when something happens to activate the virus. If this hypothesis is correct, in accordance with the theory of random sampling from a mass in which there is independent random distribution, the dilution tests should have eliminated the inactive forms, at least in a few of the plants, which would result in some cases of common mosaic without the yellow spots, unless the inactive particles are always greatly in excess of those of the common-mosaic virus. If this is true the dilution studies again should supply the evidence.

With dilutions of common mosaic of 50,000 to 100,000 times, some of the many plants which show no common mosaic should have developed late signs of yellow mosaic as a result of inactive yellow-mosaic particles which survived these dilutions, such particles later becoming active and producing infection. Yellow-mosaic spots such as are associated with common mosaic probably would not occur on these plants since the suppressing influence of the common green-mosaic virus would be absent. The yellow-mosaic symptoms in such plants should be comparable to those which develop when healthy plants are inoculated with pure virus of this mosaic. Of the many plants observed throughout the dilution studies, no such case has ever occurred. These results seemed to give satisfactory grounds for rejecting the hypothesis advanced in the preceding paragraph, but further tests were carried out to determine whether some treatment of the virus of common mosaic *in vitro* might shed light on the possibility that some type of dissociation phenomenon was operating. If such were the case striking blends of yellow and green mosaic, pure green mosaic, and pure yellow mosaic should occur in a population of plants inoculated with virus given certain treatments.

Extracts were obtained from leaves showing common mosaic without the yellow-mosaic spots. These extracts, in diluted and in undiluted form, were subjected to high temperatures (85° to 90° C.), to subfreezing, and to ultraviolet rays. Extracts were filtered and also treated with several concentrations of acid, alkali, acetone, and ethyl alcohol. Some of the extracts were first purified to a point where only slight traces of the original extraneous solids, salts, and soluble pigments were present in a water suspension of the virus. In all cases of common mosaic which resulted from virus receiving these treatments, the delayed expression of a few small yellow-mosaic spots occurred, no plants developed pure yellow mosaic, and none developed yellow-mosaic spots without the common mosaic. While many other tests might have been devised, it seemed useless to carry such methods any farther. None of the results presented point to the view that the virus of yellow mosaic is in an active or in an inactive form in each and every experimental extract of common-mosaic virus used as inoculum in the tests, but rather they favor the view that the yellow-mosaic virus arises spontaneously as a mutant.

Another line of evidence against the mixture theory is found in the results presented in table 3 and on page 962. It will be recalled that these results indicate that the viruses of type A yellow mosaic and of common mosaic are not mutually compatible in tobacco. The virus

of common mosaic is the more aggressive, as it gradually suppresses or inhibits the virus of yellow mosaic which is introduced in synthetic mixtures. This inhibiting characteristic is taken up further in the final discussion on acquired immunity, and while the phenomenon is not fully understood, it seems quite clear that it militates against or prevents the indefinite survival of the yellow-mosaic viruses thus far tested which were introduced experimentally into the virus of common mosaic. All available evidence obtained at temperatures near 21° to 24° C. indicates that unless there is a considerable quantity of yellow-mosaic virus present with the virus of common mosaic in an inoculum, the inoculated plants express symptoms of common mosaic which are indistinguishable from the symptoms expressed by the controls inoculated with the purest virus of common mosaic available (table 8). If there is sufficient virus of yellow mosaic in such a mixture to induce mild signs of yellow mosaic, these signs appear before, or simultaneously with, the first signs of common mosaic, but the subsequent leaves manifest symptoms like the controls, i. e., light- and dark-green mottling with occasional yellow-mosaic spots.

The inhibiting characteristic of the common-mosaic virus is regarded as one of the strongest bits of evidence in support of the view that the occasional yellow-mosaic spots which are persistently associated with common mosaic under the conditions of the writer's tests result not from virus introduced as a contaminant from the outside but from virus which originates as a mutant in the tissues involved in the spots. In fact, the results of all the tests presented thus far seem to support this view.

TESTS WITH A DIFFERENTIAL PLANT WHICH DEVELOPS SYSTEMIC INFECTION

It has been known for some time that certain viruses can be separated from mixtures by means of plants which are resistant to one virus but not to another. In these studies it was found that a strain of *Nicotiana glauca* served as a differential plant for the viruses of type A yellow mosaic and common mosaic in a rather unique manner at temperatures from about 21° to 24° C. in the greenhouse. When inoculated with these viruses the plants rarely developed symptoms of mosaic. When symptoms did develop they were very mild, and in the case of the yellow mosaic a few mild yellow spots occurred on 1 or 2 leaves only near the point of inoculation, the new leaves remaining mosaic-free.

It was found that a very high percentage of the *Nicotiana glauca* plants which were inoculated with the common-mosaic virus were carriers of this virus throughout their life, even though the plants manifested no visible signs of mosaic. Allard (1) reported similar results with *N. glauca* and common mosaic. In contrast with these results, it was found that none of the *N. glauca* plants which had been inoculated with the virus of type A yellow mosaic carried the virus of that mosaic in mosaic-free leaves which developed after inoculation. Thirty-five *N. glauca* plants were inoculated with the virus of type A yellow mosaic and tested in this manner, and all the tobacco plants subinoculated from them remained free of all signs of mosaic. The results of a single experiment showing the differential relationship between the viruses of common mosaic and the yellow mosaic on *N. glauca* are given in table 7.

TABLE 7.—Behavior of *Nicotiana glauca* plants when inoculated with virus of common mosaic in contrast with plants inoculated with virus of type A yellow mosaic, and the presence or absence of virus in the extracts from upper leaves of each *N. glauca* plant as shown by inoculating the extracts into tobacco plants

Virus of common mosaic used				Virus of type A yellow mosaic used ¹			
Plant no.	Presence or absence of mosaic on each <i>N. glauca</i> plant	Presence or absence of virus in each <i>N. glauca</i> plant as evidenced by plants inoculated with extracts from the upper leaves of each <i>N. glauca</i> plant 96 and 286 days after the inoculation of the latter ²		Plant no.	Presence or absence of mosaic on each <i>N. glauca</i> plant	Presence or absence of virus in each <i>N. glauca</i> plant as evidenced by plants inoculated with extracts from the upper leaves of each <i>N. glauca</i> plant 96 and 286 days after the inoculation of the latter ²	
		96 days	286 days ³			96 days	286 days ³
1.....	—	+	+	1.....	—	—	—
2.....	—	+	+	2.....	—	—	—
3.....	—	+	+	3.....	—	—	—
4.....	—	+	+	4.....	—	—	—
5.....	—	+	+	5.....	—	—	—
6.....	—	+	+	6.....	—	—	—
7.....	—	+	+	7.....	—	—	—
8.....	—	+	+	8.....	—	—	—
9.....	—	+	+	9.....	—	—	—
10.....	—	+	+	10.....	+	—	—

¹ Extracts were obtained from nearly full-sized leaves near the top of each plant of *N. glauca*.

² All plants of *N. glauca* were cut back and new shoots were allowed to develop and supply the extracts used in the second test on tobacco.

³ From 1 to 5 small yellow-mosaic spots eventually appeared on each tobacco plant that developed common mosaic.

⁴ 1 small yellow-mosaic spot appeared on 1 leaf slightly above the point of inoculation.

In another test a mixed extract obtained from equal parts of common mosaic and type A yellow-mosaic leaf tissues from tobacco was inoculated into a *Nicotiana glauca* plant. Small yellow-mosaic spots and yellowish-green spots developed on one leaf, but no other symptoms appeared on the plant. Thirty-five days after this plant was inoculated five healthy tobacco plants were inoculated with a virus extract from these spots. All of the tobacco plants developed yellow mosaic. At the same time an extract was obtained from mosaic-free leaves above the leaf with yellow-mosaic spots. This virus extract was inoculated into five healthy tobacco plants, all of which developed only common mosaic. No blends with yellow mosaic occurred, but before the plants were mature a few of the characteristic yellow-mosaic spots developed on each of them. A similar experiment was carried out with a mixture of the viruses of type A yellow mosaic and the mild dark-green mosaic. Symptoms of the mild dark-green mosaic appeared on all the plants. The healthy tobacco plants which were finally inoculated with virus from the upper leaves of these *N. glauca* plants developed only the mild dark-green mosaic; no yellow mosaic developed on any of them.

These results show that the virus of type A yellow mosaic was local in the strain of *Nicotiana glauca* used when it was grown under the conditions of the tests, and that leaves above this local region served a positive filterlike function against the virus of the yellow mosaic which was introduced in the inoculum. It will be noted in table 7 that all of the tobacco plants which were inoculated with the common-mosaic virus taken from *N. glauca* developed common mosaic.

Before maturity from 2 to 5 small yellow-mosaic spots appeared on each of the plants having common mosaic. Since no virus of yellow mosaic was recovered from the upper leaves of *N. glauca* plants which had been inoculated with virus of type A yellow mosaic, it is concluded that any virus of type A yellow mosaic which might by chance have been present in the inoculum of common mosaic used to inoculate *N. glauca*, did not reach the upper leaves of these plants. Therefore, since the few yellow-mosaic spots were delayed in their appearance on each tobacco plant, it is concluded further that the virus which induced the yellow-mosaic spots in the test tobacco plants originated in the tissue of these spots. These results are in agreement with those obtained by other methods in that they indicate virus mutation in common mosaic.

VIRUS ISOLATIONS FROM LOCAL NECROTIC LESIONS AND PRELIMINARY STUDIES ON THE LESION TECHNIQUE

The necrotic-lesion technique devised by Holmes (6) was used to throw additional light on the problem of virus mutation, and the results were reported briefly in an earlier résumé (19).

Holmes (6) suggested that necrotic lesions might be analogous to the colonies of bacteria obtained in the plating and purifying technique. Accordingly the writer used the method to determine the possibility of reclaiming the virus of mild dark-green mosaic from synthetic mixtures with the virus of type A yellow mosaic. This was done as a preliminary step as it seemed reasonable to believe the method could be applied to other viruses if satisfactory results were obtained.

Leaves of *Nicotiana glutinosa* were removed from the plants, wiped with virus extract, and thoroughly rinsed with sterile distilled water. The leaves were then placed in a previously sterilized covered dish and kept in a humid atmosphere at about 22° C. until the necrotic lesions developed, great care being taken to maintain aseptic conditions, so far as foreign viruses were concerned, during the whole procedure. The lesions were examined under a lens in order to discard any cases in which incipient lesions might be close to the typical lesions. Lesions sufficiently separated were carefully removed by means of a small sharp dissecting spear, and each lesion was macerated and pushed into the junction point of a petiole and stem of a mosaic-free tobacco plant.

Nineteen lesions were selected at random from leaves wiped with the above-mentioned mixture of viruses. Each lesion was used to inoculate a single tobacco plant. Two of these plants developed yellow mosaic, 6 developed typical mild dark-green mosaic with no signs of yellow mosaic, and the remaining 11 were mosaic-free. Since the virus of mild dark-green mosaic was recovered free from the virus of yellow mosaic, it was concluded that this method serves to separate mixed viruses.

Another test was carried out with the virus of common mosaic. This extract was obtained from tobacco leaves showing no signs of yellow-mosaic spots. It was diluted in water to reduce the number of lesions and used to wipe the leaves removed from *Nicotiana rustica* and *N. glutinosa* plants. One hundred and twenty-seven lesions were isolated from these leaves and inoculated separately into the same number of young tobacco plants. Of these plants, 120 developed

typical common mosaic, and typical yellow-mosaic spots eventually made their appearance on each plant. The 7 mosaic-free plants remained so during the test. The appearance of the yellow-mosaic spots was delayed, and 1 to 3 spots appeared on each plant as is usual in common mosaic at moderate growing temperatures.

As in all previous tests, the results obtained with the local-lesion technique indicate that the virus of the yellow-mosaic spots does not represent a contamination from outside sources which is carried along in all inoculation extracts of common mosaic, and they support the idea of virus mutation. This conclusion is based on the successful separation of the virus of mild dark-green mosaic from the virus of yellow mosaic, and not on the assumption that necrotic lesions are incited by single virus particles.

This paper deals primarily with the problem of virus mutation and not with the necrotic-lesion technique. However, since this method is used in virus-mutation studies, it seems permissible to digress from the main subject long enough to discuss certain aspects of this technique.

It has been considered (8, 10, 23) that the local lesions rarely contain more than one type of virus when mixed viruses are used to wipe the leaves of suitable species, and it is inferred that the lesions are induced for the most part by single virus particles. So far as the writer knows, no data have been presented which make it possible to pass fair judgment on this point. Mixed extracts containing the viruses of common mosaic and yellow mosaic have been used to wipe leaves of species which produce local necrotic lesions. The resulting necrotic lesions have been isolated and each has been inoculated into a single tobacco plant, with the result that the infected plants developed either yellow mosaic or common mosaic, but rarely blended symptoms of both diseases. On this basis it has been considered that necrotic lesions are rarely incited by more than a single virus particle.

This method of determining the presence of type A yellow-mosaic virus in a lesion which also contains the virus of common mosaic is of questionable value for the reason that all the evidence indicates that the virus of common mosaic is more aggressive than that of type A yellow mosaic. It is admitted that this evidence was obtained from systemic symptoms, but until otherwise shown, it is reasonable to suppose that the same aggressive relationship obtains in lesions.

The data presented in table 3 rather clearly indicates that this aggressive characteristic prevented the expression of yellow-mosaic symptoms in many tobacco plants which were inoculated with yellow-mosaic virus extract that had been diluted 100, 500, and 1,000 times in virus extract of common mosaic. All the plants that were inoculated with mixtures containing the virus extract of yellow mosaic in quantities greater than the virus extract of common mosaic first developed the symptoms of pure yellow mosaic, and these persisted for some time. During this period the experimenter would conclude that these plants had received no virus of common mosaic in the inoculum, and if the plants had been discarded during this period it is evident from the tables that this conclusion would be erroneous. On long standing, each of these plants eventually gave the true story. Even though the quantity of common-mosaic virus was smaller in the

beginning, its aggressive and suppressive characteristics made possible the manifestation of common mosaic before the plants were mature.

The tests cited in tables 3 and 4 were made some time before the local-lesion method was developed. However, after Kunkel (11) reported that *Nicotiana sylvestris* developed necrotic lesions when inoculated with virus of aucuba mosaic (a yellow mosaic), but systemic symptoms when inoculated with the virus of common mosaic, the following tests were carried out. These tests were made to determine further the possibility of getting a satisfactory assay for yellow-mosaic virus in mixtures with the virus of common mosaic when using tobacco as the test plant.

Fresh extracts were obtained from actively growing leaves on tobacco plants with type A yellow mosaic and also from plants with common mosaic. These extracts were mixed in the proportion of 1 part to 99 parts and 1 part to 999 parts, respectively. In addition, there were controls in which water was used to dilute the virus of yellow mosaic. Each of these extracts, including the undiluted extracts, was used to inoculate 10 healthy tobacco plants by the needle-cotton method described previously (14), to inoculate 10 healthy tobacco plants by wiping 1 medium-sized leaf on each plant, and to wipe 10 or more leaves on *Nicotiana sylvestris* plants. The total leaf area wiped in a given set of 10 tobacco plants was estimated to be about equal to the total leaf area wiped on the *N. sylvestris* plant inoculated with the corresponding extract. The leaf area wiped on each plant of *N. sylvestris* averaged approximately 1,000 cm². The leaves of *N. sylvestris* were wiped after the tobacco plants were inoculated.

The tests on *Nicotiana sylvestris* (table 8) show that virus of yellow mosaic was potent in the mixtures with common-mosaic virus which were inoculated into the tobacco plants, and the tests on tobacco with the yellow-mosaic virus which was diluted in water show a high percentage of infection. It will be observed further that no striking case of yellow mosaic appeared in any of the tobacco plants inoculated with the extracts containing the viruses of both mosaics. A few plants showed yellow-mosaic spots on one or more of the first leaves. The great majority of plants developed common mosaic which was indistinguishable from that in the common-mosaic controls. Dilutions of 100 and 1,000 are not great, yet these tests demonstrated that it was not possible by the systemic-symptom method alone to obtain an adequate test for the yellow-mosaic virus which was present in appreciable amounts with the virus of common mosaic. However, subsequent tests have shown that type A yellow-mosaic virus which was present in similar plants with common mosaic was detectable by means of necrotic lesions on the leaves of *N. sylvestris* which had been wiped with the extracts from such plants.

Kunkel (10) used *Nicotiana sylvestris* as a test plant for viruses from lesions produced on leaves of *N. langsdorffii* Schrank., which had been punctured with needles carrying mixed viruses of common mosaic and aucuba mosaic. No data were presented, but from these tests he concluded that except in a small percentage of cases the lesions resulted from a single unit of virus. His use of the term "unit" is not defined, but one is left to infer that it signifies one particle and not a minimum dose, which might include several particles.

TABLE 8.—Results of 2 tests illustrating the uncertainty of determining virus mixtures of common mosaic and type A yellow mosaic at 21° to 24° C.

Parts of virus extract or water used in preparing the mixtures			Method used for making assay ¹	Notes on tobacco plants based on observations made during the first 30 days after inoculation
Water	Extract of common mosaic	Extract of yellow mosaic		
0	0	1	A.----- B.----- C.-----	10 tobacco plants had yellow mosaic. Do. 2.91 necrotic lesions per cm ² of leaf surface on <i>Nicotiana sylvestris</i> .
99	0	1	A.----- B.----- C.-----	10 tobacco plants had yellow mosaic. Do. 0.117 necrotic lesions per cm ² of leaf surface on <i>N. sylvestris</i> .
0	99	1	B.----- C.----- A.-----	6 tobacco plants had common mosaic only; 4 tobacco plants had common mosaic predominating, but yellow-mosaic spots appeared on from 1 to 4 of the first leaves to show mottling. 6 tobacco plants had common mosaic only; 4 tobacco plants had common mosaic predominating, but yellow-mosaic spots appeared on from 1 to 3 of the first leaves to show mottling. 0.098 necrotic lesions per cm ² of leaf surface on <i>N. sylvestris</i> .
0	1	0	A.----- B.----- C.-----	10 tobacco plants had common mosaic. Do. No necrotic lesions on leaf surface of <i>N. sylvestris</i> .
0	0	1	A.----- B.----- C.-----	10 tobacco plants had yellow mosaic. Do. 1.03 necrotic lesions per cm ² of leaf surface on <i>N. sylvestris</i> .
999	0	1	A.----- B.----- C.-----	6 tobacco plants had yellow mosaic; 4 tobacco plants mosaic-free. 10 tobacco plants had yellow mosaic. 0.014 necrotic lesions per cm ² of leaf surface on <i>N. sylvestris</i> .
0	999	1	A.----- B.----- C.-----	10 tobacco plants had common mosaic. 10 tobacco plants had common mosaic, but 1 plant had 1 yellow-green spot on the second leaf which developed common mosaic. 0.022 necrotic lesions per cm ² of leaf surface on <i>N. sylvestris</i> .
0	1	0	A.----- B.----- C.-----	10 tobacco plants had common mosaic. Do. 0.006 necrotic lesions per cm ² of leaf surface on <i>N. sylvestris</i> . ²

¹ 3 methods of testing were used as indicated by the roman letters as follows: A, Tobacco plants were inoculated by the needle-cotton method previously described (14); B, tobacco plants were inoculated by wiping a leaf with the same extract used in method A; C, 10 or more leaves of *N. sylvestris* plants were inoculated by wiping with the same extract used in method A. The number of necrotic lesions is based on a unit area of the leaf surface, i. e., 1 cm².

² The common-mosaic tissue used for this control contained a few yellow-mosaic spots.

The writer has not had an opportunity to test the amount of increase of virus in the lesions produced by *Nicotiana langsdorffii*. However, tests have indicated that the increase frequently is very small in necrotic lesions produced by *N. rustica*, *N. glutinosa*, and *N. sylvestris*. Caldwell (2) also found a small increase of the virus in the necrotic lesions he tested. It is a common occurrence to get negative results in testing for the presence of virus in necrotic lesions, and with common-mosaic virus it has been unusual to obtain more than 10 lesions on a leaf of *N. rustica* when wiped with extract from a single lesion isolated from *N. rustica*, the leaves in both instances being attached to the plant. This situation makes it difficult to obtain a positive test for the presence of one or the other virus in a mixture if one virus is considerably in excess of the other or if one is decidedly more aggressive than the other. Tests indicate that the central areas in lesions contain little or no virus when they become necrotic; thus in many cases the evidence of a primary mixture might not be obtained when only the peripheral virus is available for test.

If it is found that necrotic lesions in the great majority of cases are incited by single virus particles, it will be of considerable experimental importance. However, wholly aside from the point as to the actual number of virus particles which incite a lesion, it is reasonably evident

that certain necrotic lesions either contain but one type of virus, or in some cases of mixture, one virus is of such high relative concentration that the presence of others in the lesion cannot be detected by ordinary methods. Consequently those in the minority which are not too aggressive are completely lost in series transfers, leaving the most concentrated virus to survive in a pure state.

While the lesion method does permit the separation and purification of certain viruses, it should be made very clear that the techniques which are based on the systemic invasion of the virus and mosaic mottling have made it possible to separate and purify many viruses through the isolation of local tissues which exhibit departures in symptoms, followed by the dilution of the virus extract thus obtained and its subsequent inoculation into populations of plants. This procedure is based on the elementary principle of independent random distribution of the particles in an extract and on reducing the concentration of the virus that is present in smaller quantity to a point where it is no longer detectable in all of the random samples used for inoculating the test population. Through a careful study of symptoms the worker learns to detect irregularities in local areas of tissue in a given plant and in individual plants in a series. Selections and inoculations from these through successive tests frequently establish new types of virus. This method has wide application and can be used in studying certain viruses not known to induce the local lesions.

TESTS WITH DIFFERENTIAL PLANTS WHICH DEVELOP LOCAL NECROTIC LESIONS

In studies on *Nicotiana* species a collection of *N. affinis* was received which comprised several rather obvious genetic types differing in flower color, time of flowering, and texture of leaves. In the group which produced flowers late it was found that necrotic lesions developed on the detached leaves which were wiped with the virus of type A yellow mosaic. In certain of these plants necrotic lesions developed on detached leaves which were wiped with virus of common mosaic, but in other plants the wiped leaves developed yellowish-green areas. Kunkel (11) has reported that the virus of common mosaic fails to induce necrotic lesions, whereas the virus of aucuba mosaic (a yellow mosaic) does induce necrotic lesions on the leaves of *N. sylvestris*.

This differential relationship in *Nicotiana affinis* and *N. sylvestris* was used in testing the virus-mutation theory in the following ways. Plants were first tested to determine whether their leaves were negative for the expression of the characteristic necrotic lesions when wiped with virus extract of common mosaic. This was done with detached leaves to insure against systemic infection in order that a given plant might be used to supply leaves for several experiments. Another set of detached leaves was wiped with a synthetic mixture of common-mosaic virus and type A yellow-mosaic virus in the proportion of 999 parts to 1, and it was found that the yellow-mosaic virus did induce necrotic lesions.

When plants were known to be satisfactory for test purposes, 4 leaves were removed, 3 from *Nicotiana affinis* and 1 from *N. sylvestris*, and each was wiped with a virus obtained from a different tobacco plant having common mosaic. No necrotic lesions developed on these leaves. Each leaf was used to inoculate healthy tobacco plants. In all, 18 plants were inoculated. All plants developed common mosaic, and the delayed expression of from 1 to 4 small yellow-mosaic spots

occurred on each plant. Three of these spots were tested on *N. sylvestris* and the viruses were found to induce necrotic lesions.

Another test was carried out with a plant of *Nicotiana sylvestris* known to be a positive tester for the lesions of type A yellow mosaic when in combination with the virus of common mosaic. One leaf of this plant was trimmed, wiped with extracts of common mosaic and of yellow mosaic, and rinsed with water. The method is illustrated in figure 4. Care was exercised to confine the virus extracts to the areas wiped. The leaf was left attached to the plant. Necrotic lesions appeared on the margin wiped with virus of yellow mosaic. No necrotic lesions appeared on the half of the leaf wiped with virus of common mosaic, but yellowish-green areas did appear and common mosaic finally developed in the new leaves. As soon as mosaic appeared in the young leaves the inoculated leaf was removed. The portion of this leaf lying between the midrib and near the border of the necrotic lesions was cut into two strips, sections *b* and *c* as shown in figure 4, and each strip was pulped in a small amount of water. The resulting inoculum from each strip was wiped separately on attached leaves of two plants of *N. sylvestris*. All these leaves remained free of lesions. Common mosaic appeared on the new leaves of the plant inoculated with extract from the strip nearest to the midrib, but the plant inoculated with extract from the other strip remained healthy. A detached leaf from each of these plants was wiped with virus of type A yellow mosaic and typical lesions appeared, showing that the plants were of suitable test value.

These results seem to support the view that the virus of type A yellow mosaic did not pass from the leaf illustrated in figure 4 into the new leaves. The small central leaves showing common mosaic were removed and pulped. The resulting extract was wiped on leaves of *Nicotiana sylvestris* and also inoculated into tobacco plants. No necrotic lesions developed on the leaves of *N. sylvestris* but common mosaic did develop. Only the green mottling of common

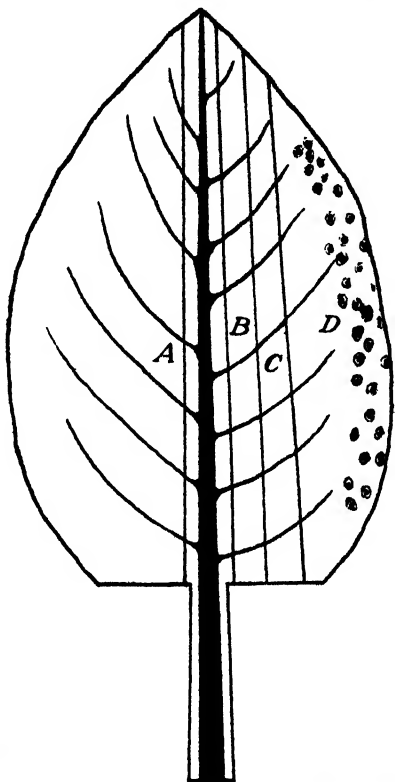


FIGURE 4.—Diagram of a leaf of *Nicotiana sylvestris* illustrating a method of inoculation used to determine whether the virus of type A yellow mosaic is detectable in the virus extract of common mosaic, and whether the virus of this yellow mosaic passed into tissues far removed from the lesions. The leaf was left attached to a healthy plant and inoculated in the following manner: The base was trimmed as shown, section *a* was then wiped with a virus extract of common mosaic and section *d* was wiped on the margin with virus extract of yellow mosaic. No necrotic lesions developed in section *a*, but many lesions developed on the margin of section *d*. When common mosaic appeared in the small central leaves of the plant the inoculated leaf was removed. Sections *b* and *c* were cut from the leaf and each was pulped separately, and the resulting extracts were wiped on leaves of separate plants of *N. sylvestris*. No necrotic lesions appeared on these leaves, but common mosaic appeared in the plant which was wiped with the extract from section *b*. The plant wiped with extract from section *c* remained mosaic-free.

mosaic appeared on the plants during the first 4 weeks of growth, but after that a few small yellow-mosaic spots appeared on each plant.

Virus extracts were obtained from some of these yellow-mosaic spots and wiped on the leaves of *N. sylvestris*. In some cases only the typical necrotic lesions developed and in other cases a systemic type of yellow mosaic resulted. The systemic type of yellow mosaic was not in evidence in previous tests of the stock virus of type A yellow mosaic.

The results obtained in the tests with *N. sylvestris* and *N. affinis* seem to warrant the conclusion that the yellow-mosaic spots which appeared in the test tobacco plants resulted from viruses which originated in the tissues involved in the spots and not from virus which had been carried along as a contaminant with the virus of common mosaic. As in all other methods used, this method supports the view that the virus of common mosaic mutates.

TYPES OF YELLOW MOSAIC ISOLATED FROM TOBACCO

It was pointed out in an abstract (17) that several types of yellow mosaic have been isolated. Three of these are described briefly here.

Type A.—This yellow mosaic resembles Johnson's (9) virus no. 6. It was isolated by the writer from the common mosaic which was used throughout the present studies. At high summer temperatures tobacco plants develop a bright yellow or sometimes a creamy white mottling. When growing temperatures are reduced the yellow color of the mottled areas of subsequent foliage gives way to a light green as a result of a gradual increase in the chlorophyll content. At temperatures of about 13° to 15° C., this yellow mosaic is indistinguishable from the common mosaic on tobacco plants grown at temperatures of about 21° to 24°, yet the virus from plants grown at these low temperatures induces typical yellow mosaic when inoculated into plants cultured at 21° to 24°, thus indicating that the symptom changes are not due to a mixed virus.

On the Canary Island strain of *N. glauca*, symptoms are either absent or very mild, especially at 21° C. and below, and the virus is not carried in mosaic-free leaves and stems in the upper portion of plants that are allowed to continue growth for several weeks or months after inoculation.

The virus produces necrotic lesions on the leaves of *N. rustica*, *N. glutinosa*, *N. sylvestris*, and certain strains of *N. affinis* at 21° to 24° C. The properties of the virus in vitro are similar to those of the virus of common mosaic.

If the strain of *N. glauca* used by Johnson (9) is genetically the same as the writer's strain, then type A yellow mosaic may be the same as Johnson's no. 6 yellow mosaic, for he states that the symptoms are often masked on *N. glauca*. Jensen (8) has described a yellow mosaic (isolation no. 102) that shows symptoms like the writer's type A yellow mosaic on tobacco, and he considers his mosaic to be similar to Johnson's no. 6 and to aucuba mosaic, but, according to Kunkel (11) aucuba mosaic induces necrotic lesions on leaves of *N. sylvestris*, whereas Jensen's does not.

Type B.—This yellow mosaic was isolated from a yellow spot associated with a green mosaic on tobacco. The original virus was in *Nicotiana glauca* collected on the island of Grand Canary. The

mild dark-green mosaic referred to in this paper predominated in this material and a common mosaic was also isolated with the yellow spot. At high summer temperatures this mosaic on tobacco has been indistinguishable from type A. However, chlorophyll masks the yellow areas of type B mosaic more rapidly at lower temperatures than it does those of type A. At temperatures near 15° to 19° C. the symptoms of type B mosaic on tobacco resemble those of common mosaic on tobacco when grown near 21° to 24° C.

On the Canary Island strain of *N. glauca* this mosaic is characterized by very severe yellow mottling on all leaves, stems, and petioles, and the virus is systemic in all these parts.

The virus produces necrotic lesions on the leaves of *N. rustica*, *N. glutinosa*, *N. sylvestris*, and certain strains of *N. affinis* at 21° to 24° C. The lesions resemble those induced by the virus of type A. The properties of the virus in vitro are similar to those of the virus of common mosaic.

If the strain of *N. glauca* used by Johnson is genetically the same as the writer's strain, then type B yellow mosaic appears to differ from Johnson's no. 6 yellow mosaic, as the writer has always obtained severe symptoms at temperatures above 21° C. on this host. Strain differences in this host must be reckoned with in virus studies, as the writer's tests indicate that mottling is more severe on the Canary Island strain than on the Babcock and Clausen strains.

Type C.—This yellow mosaic was isolated from a yellow spot associated with a green mosaic resembling common mosaic on tobacco. The original virus was in *Nicotiana glauca* collected by the writer on the island of Grand Canary. The yellow pattern of this mosaic on tobacco is distinct from that caused by types A and B. The designs take on the appearance of lace and the degree of yellowing is less than in types A and B. The virus of this mosaic does not produce symptoms on tomato, and tomato is not a carrier of the virus. On the Canary Island strain of *N. glauca* the symptoms appear on all leaves, but they are less severe than those induced by type B. The virus produces necrotic lesions on the leaves of *N. rustica*, *N. glutinosa*, and *N. sylvestris* at 21° to 24° C. The virus retains its potency in dry tissue for at least 29 months at room temperatures. The thermal destruction point of the virus has been between 80° and 85° C. in several tests.

During the process of purifying this virus it was found that a mild dark-green mosaic was associated with it. This green mosaic is very similar to, if not identical with, the mild dark-green mosaic used in the experiments cited in this paper. Since the virus of type C yellow mosaic possesses several properties in common with the virus of mild dark-green mosaic, it is suspected that this yellow-mosaic virus may have originated from the virus of mild dark-green mosaic under special conditions not yet maintained in the writer's experiments.

DISCUSSION

It has been shown that a few small yellow spots occurred in all the collections of common mosaic studied. After the isolation and purification of the viruses from these spots it was found throughout long series of subinoculations that the viruses induce symptoms which are a distinct departure from those of common mosaic.

Tests indicate that these viruses arise spontaneously in association with common mosaic and not from contaminations from outside sources and the evidence presented seems to support the view that these new viruses represent mutants and not loosely fixed variants.

The precise mechanism involved in the spontaneous origin of one virus from another is not known. Assuming the virus to be living and capable of independent regeneration, the process may be similar to the saltation or mutation in fungi and in higher organisms. However, it seems likely that the virus is no larger than, if as large as, some genes, and therefore the mutation would involve a very minute physical-chemical mechanism as in the case of genes. It is possible that the process involves changes in a chemical compound due to the addition of or the dropping of a radical, to alterations in valence, or to isomeric changes. If the plant cells provide a special mechanism for increasing the virus, it is possible that the first step in the mutation of the virus consists of a change in the function of the virus-producing mechanism.

The fact that yellow-mosaic spots appeared on all of the many plants with common mosaic might be advanced as an argument against the mutation of the virus on the ground that the occurrence is too frequent. However, this argument carries little weight for the reason that the frequency must be based on the total number of virus particles involved in all of the plants and not on the number of plants. On this basis the millions of virus particles involved in a single plant would indicate a low frequency at the temperatures employed in these tests.

The expression of the yellow-mosaic spots seems to be influenced by the environment; therefore this factor must be reckoned with in studies on this problem. It is entirely possible that some of the viruses and hosts found suitable for these studies might not be suitable under environmental conditions differing markedly from those maintained in this work. Host material which is suitable for developing evidence on the virus-mutation theory with certain yellow mosaics and common mosaics of tobacco may be unsuitable for another combination. For example, *Nicotiana glauca* was satisfactory for type A yellow mosaic and the common mosaic associated with it, but not for type B yellow mosaic and the common mosaic associated with it because both of these latter viruses induced symptoms and were systemic in *N. glauca*. Heterozygosity in test plants must be kept in mind constantly in virus-mutation and classification studies. This was especially evident in the studies involving *N. affinis*. In critical work where a considerable number of plants are needed it is sometimes necessary to propagate vegetatively from single plants in order to reduce genetic irregularity to a minimum.

In addition to the 3 yellow mosaics isolated in these studies, Jensen (8) isolated 3 yellow mosaics from common mosaic of tobacco, and Price (23) isolated several yellow mosaics from cucumber mosaic. In wheat the writer (18) reported the isolation of a yellow mosaic which is closely associated with a green mosaic, and later he⁷ reported another yellow mosaic which is closely associated with another green mosaic. Since that time another similar association

⁷ McKINNEY, H. H. WHEAT MOSAIC IN KANSAS. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rept. 16: 115-116. 1932. [Mimeographed.]

has been found. These yellow mosaics in wheat may originate in the same manner as those in tobacco, but the evidence is less readily obtained. However, in the case of the green-mosaic-rosette virus repeated isolations from green mottled tissues for 5 years have failed to eliminate all traces of the yellow mosaic, leaving one to infer that the mutable characteristic is common to the green-mosaic rosette of wheat.

A foliage-deforming green mosaic has been isolated from a light-green spot associated with the mild dark-green mosaic used in these studies.

In addition to the severe yellow mosaics which arise from common mosaic, it appears from the results presented by Holmes (?) that a certain masked mosaic arises from a mosaic which induces mottling.

Virus mutation opens up an interesting and important field dealing with the origin of new types, the evolutionary development of viruses, and their relationships. The new types obtained thus far from established types appear to possess many properties in common with the virus from which they are considered to have originated. The most striking departure of a new type thus far has been in the relative intensity of the symptoms induced in the host, accompanied by relatively minor changes in host range and other properties. Minor changes in properties may signify only strain differences, but regardless of this point there seems to be ample justification for considering that another virus has been isolated even though it may show a consistent departure from the original in but one property or characteristic. As this field of study is opened up it seems entirely possible that viruses may be obtained which will show even more extreme departures from the originals. A virus manifesting a radical departure might arise through a single mutation, but it seems more likely that extreme changes in properties and in host range are eventually effected as mutation proceeds through several stages involving different varieties and species of plants growing in different environments.

The virus of tobacco common mosaic and the virus of cucumber common mosaic may be thought of as representing two distinct central or basic types, each fixed within limits but capable of producing by mutation its particular types or strains which in turn may mutate. While the writer is inclined to the view that the mutants from these two viruses will probably fall for the most part into two distinct groups or ranges, it is possible that some of the mutants in the two groups may be very similar to each other or even overlap in certain characteristics. From the studies on the tobacco common mosaic the writer is inclined to the view that this virus is not fixed to the extent that most workers believe. It is strongly suspected that some of the collections of common mosaic show differences of a magnitude which are fully as great as the differences exhibited between some of the yellow mosaics that arise as mutants from common mosaic. It seems entirely possible that the virus of tobacco common mosaic may have originated from another virus which may or may not induce mottling symptoms in tobacco. It is not beyond the realm of possibility that there may be viruses which cannot induce any of the now recognized symptoms in any known species. However, mutants from such viruses might be capable of inducing signs of disease.

It seems probable that several strain types of virus resulting from mutation are present in many tobacco plants having common mosaic, and that this may occur in many virus diseases. Such a complex may explain the so-called attenuation and the increased virulence attributed to the exposure of certain viruses to extreme temperatures, passages of virus through different plants or animals, and to other treatments.

The role of mutation in epidemiology has several angles which should be considered. Throughout the studies at the Arlington farm, the pure yellow mosaics of tobacco have been handled under quarantine, and all diseased plants and the soil in which they grew have been sterilized with steam before being disposed of. On tobacco and tomato the yellow mosaics are much more destructive than green mosaic, and with the exception of the green-mosaic rosette on Harvest Queen wheat and similar varieties, this is true among susceptible varieties of wheat. On the other hand, there are certain natural factors which tend to restrict certain of the yellow mosaics.

It was pointed out in an earlier paper (15) that pure yellow mosaics occur less generally than green mosaic on *Nicotiana glauca* in the Canary Islands. The same was found to be true in commercial fields of tobacco visited in Pennsylvania, Virginia, and Maryland. This is explained on the basis that there are few sources of pure yellow mosaic to serve as centers for spread. These are few because of the extremely small possibility of transmitting pure yellow mosaic from the relatively small number of yellow spots that appear on plants having common mosaic. Measurements made on a typical tobacco plant with common mosaic showed that the total leaf area was approximately 13,600 cm², and in this area about 1 cm² was involved in yellow-mosaic spots. From this it is clear that natural and cultural factors could rarely be expected to successfully establish pure yellow mosaic in another plant from the small spots. On the other hand, the chances are essentially reversed when plants with pure yellow mosaic are exposed to natural factors in a field.

The characteristic action of common-mosaic virus in gradually suppressing or inhibiting at least certain yellow-mosaic viruses arising from it doubtless plays an important role in delimiting the size of the yellow-mosaic spots. This characteristic also militates against the rapid establishment of cases of pure yellow mosaic under natural conditions. However, if these yellow mosaics get into new areas or on new varieties where the suppressing influence may be reduced or possibly reversed, they are likely to be very destructive.

The suppressing influence of one virus over another is not fully understood, but it may be that the phenomenon represents differences in the ability of the viruses to compete in their development, a situation which may exist without reference to a special defensive mechanism set up by the plant. It seems entirely possible that this phenomenon is similar to the antagonism between certain fungi and between certain bacteria.

Kunkel (11) in his study of the yellow-mosaic and common-mosaic viruses on *Nicotiana sylvestris* concluded that the virus of common mosaic induces an acquired immunity in the plant against the virus of yellow mosaic. In similar studies Caldwell (3) referred to the phenomenon as induced immunity. The general conception of ac-

quired immunity has grown up to a very large extent around the classic example of immunization against smallpox by a presumed special defense mechanism set up in the body as a result of the immunizing agent. However, a review of the literature on acquired immunity in mammals shows that there are a number of types which differ to a considerable extent from the situation obtaining in acquired immunity to smallpox, and that acquired immunity is relative to a considerable degree.

The writer's tests have shown that the common mosaic gradually suppresses the yellow-mosaic virus in the meristematic tissues, thereby effecting what possibly may be considered a cure for yellow mosaic. Leaves already displaying yellow mosaic continued to show it until they died, but before the end of the growth cycle of the plant the yellow mosaic was to all intents and purposes cured in the leaves that were produced after those in which the blended symptoms appeared. Furthermore, common mosaic developed on side branches from the axils of leaves which had expressed yellow mosaic before the reinoculation with virus of common mosaic. Preliminary studies have indicated that the suppression of the yellow-mosaic symptoms is more rapid than the suppression of the virus. In the early stages of recovery from yellow mosaic the yellow-mosaic virus is present in considerable amount in leaves showing only the light- and dark-green mottling of common mosaic.

This so-called "curative characteristic" is not an outstanding attribute of the agents which immunize against virus diseases in mammals, though in the case of rabies the disease is prevented when the Pasteur treatments are administered before the completion of the incubation period. Alleviating effects have been claimed for some of the anti-toxins and for the antiserum used against cerebrospinal meningitis.

It is true that tobacco plants inoculated with the virus of common mosaic are protected against the occurrence of pure yellow mosaic, but the symptoms of common mosaic remain and the disease persists throughout the life of the plant when grown under normal culture conditions. This characteristic is distinctly at variance with the situation found in acquired immunity among mammals.

If we hold strictly to the prevailing interpretation of acquired immunity as exemplified in mammals, it is difficult to consider the mosaic relationship in question as representing bona fide acquired immunity. On the other hand, it is quite possible that the prevailing interpretation is too limited for the purpose of the plant pathologist, and that the virus of common mosaic should be looked upon as an immunizing agent or "vaccine" which protects the tobacco plant against the yellow mosaic. It seems unnecessary to assume that the virus of common mosaic induces the plant to set up a special protective mechanism, such as a system of antibodies. It appears also that the common-mosaic virus represents a rather low form of "vaccine" since the disease produced by it is permanent. On the other hand, the virus represents a relatively high form of "vaccine" since ultimately it renders a "cure." The *G* virus used in Salaman's (24) tests against the *L* virus in tobacco and *Datura stramonium* is a more efficient "vaccine" since it induces very slight symptoms with no appreciable effect on the health of the plants. Simon (25) has pointed out in connection with the control of smallpox that during the early period

when prophylaxis was obtained through variolation certain people so treated served as carriers of the smallpox virus though they were immunized against the disease. This danger was not removed until the cowpox vaccine method was used. In the case of tick fever, cattle which have completely recovered from an attack of the disease carry the parasite in the blood for long periods.

In view of the mutable nature of some of the plant viruses, it is possible that in time viruses may be isolated which will protect as well as "cure" and yet will not survive indefinitely in an active form in the plant.

SUMMARY

Twenty-three collections of viruses which induce common mosaic or similar types of green mosaic on tobacco were obtained from different parts of the world. All of these mosaics developed a few bright yellow or yellowish-green spots of small size on the foliage of each tobacco plant tested. Three other viruses which induce green mosaics distinctly not of the common type on tobacco did not induce these yellow-mosaic spots.

These spots contain viruses which are distinct from the virus of common mosaic.

After the isolation and purification of viruses from these spots it was found through a long series of subinoculations that new symptoms are consistently associated with the new viruses. Thus the new viruses represent permanent departures from the established type, the essential criterion for mutation.

The yellow-mosaic viruses from the common mosaic do not compete successfully with the virus of common mosaic in tobacco. When both viruses are introduced into the plant in approximately equal parts yellow mosaic develops, but the virus of common mosaic gradually predominates until the symptoms of common mosaic take possession of the top leaves. The same results obtain when plants with yellow mosaic are reinoculated with the virus of common mosaic, but when plants with common mosaic are reinoculated with the virus of yellow mosaic no changes are apparent in the symptoms.

LITERATURE CITED

- (1) ALLARD, H. A.
1917. FURTHER STUDIES OF THE MOSAIC DISEASE OF TOBACCO. *Jour. Agr. Research* 10: 615-632, illus.
- (2) CALDWELL, J.
1932. STUDIES IN THE PHYSIOLOGY OF VIRUS DISEASES OF PLANTS. III. AUCUBA OR YELLOW MOSAIC OF TOMATO IN NICOTIANA GLUTINOSA AND OTHER HOSTS. *Ann. Appl. Biol.* 19: 144-152, illus.
- (3) ———
1935. ON THE INTERACTION OF TWO STRAINS OF A PLANT VIRUS; EXPERIMENTS ON INDUCED IMMUNITY IN PLANTS. *Roy. Soc. [London], Proc., Ser. B* 117: 120-139, illus.
- (4) DUFRENOY, J.
1933. DIFFERENTIATION OF GREEN- AND YELLOW-MOSAIC VIRUSES IN TOBACCO. (Abstract) *Phytopathology* 23: 10.
- (5) HOGGAN, I. A.
1935. TWO VIRUSES OF THE CUCUMBER MOSAIC GROUP ON TOBACCO. *Ann. Appl. Biol.* 22: 27-36, illus.
- (6) HOLMES, F. O.
1929. LOCAL LESIONS IN TOBACCO MOSAIC. *Bot. Gaz.* 87: 39-55, illus.
- (7) ———
1934. A MASKED STRAIN OF TOBACCO-MOSAIC VIRUS. *Phytopathology* 24: 845-873, illus.

-
- (8) JENSEN, J. H.
1933. ISOLATION OF YELLOW-MOSAIC VIRUSES FROM PLANTS INFECTED WITH TOBACCO MOSAIC. *Phytopathology* 23: 964-974, illus.
- (9) JOHNSON, J.
1927. THE CLASSIFICATION OF PLANT VIRUSES. *Wis. Agr. Expt. Sta. Research Bull.* 76, 16 pp., illus.
- (10) KUNKEL, L. O.
1934. TOBACCO AND AUCUBA-MOSAIC INFECTIONS BY SINGLE UNITS OF VIRUS. (Abstract) *Phytopathology* 24: 13.
- (11) ———
1934. STUDIES ON ACQUIRED IMMUNITY WITH TOBACCO AND AUCUBA MOSAICS. *Phytopathology* 24: 437-466, illus.
- (12) MCKINNEY, H. H.
1926. VIRUS MIXTURES THAT MAY NOT BE DETECTED IN YOUNG TOBACCO PLANTS. (Phytopath. Note) *Phytopathology* 16: 893.
- (13) ———
1927. FACTORS AFFECTING CERTAIN PROPERTIES OF A MOSAIC VIRUS. *Jour. Agr. Research* 35: 1-12.
- (14) ———
1927. QUANTITATIVE AND PURIFICATION METHODS IN VIRUS STUDIES. *Jour. Agr. Research* 35: 13-38, illus.
- (15) ———
1929. MOSAIC DISEASES IN THE CANARY ISLANDS, WEST AFRICA, AND GIBRALTAR. *Jour. Agr. Research* 39: 557-578, illus.
- (16) ———
1930. A MOSAIC OF WHEAT TRANSMISSIBLE TO ALL CEREAL SPECIES IN THE TRIBE HORDEAE. *Jour. Agr. Research* 40: 547-556, illus.
- (17) ———
1931. FOUR APPARENTLY UNDESCRIBED MOSAICS WHICH GO TO TOBACCO. (Abstract) *Phytopathology* 21: 118.
- (18) ———
1931. DIFFERENTIATION OF VIRUSES CAUSING GREEN AND YELLOW MOSAICS OF WHEAT. *Science (n. s.)* 73: 650-651.
- (19) ———
1931. ÉTUDE SUR LES MÉLANGES DE VIRUS. 2 Cong. Internatl. Path. Compar., Paris *Compt. Rend. et Commun.* 2, pp. 449-453, illus.
- (20) NOLLA, J. A. B., GUGGENHEIM, J. S., and ROQUE, A.
1933. A VARIETY OF TOBACCO RESISTANT TO ORDINARY TOBACCO MOSAIC. *Jour. Dept. Agr. Puerto Rico* 17: 301-303.
- (21) PETERSON, P. D.
1931. PLASTID PIGMENT AND CHLOROPHYLLASE CONTENTS OF TOBACCO PLANTS AS INFLUENCED BY THREE TYPES OF MOSAIC. (Abstract) *Phytopathology* 21: 119.
- (22) PRICE, W. C.
1933. THE THERMAL DEATH RATE OF TOBACCO-MOSAIC VIRUS. *Phytopathology* 23: 749-769, illus.
- (23) ———
1934. ISOLATION AND STUDY OF SOME YELLOW STRAINS OF CUCUMBER MOSAIC. *Phytopathology* 24: 743-761, illus.
- (24) SALAMAN, R. N.
1933. PROTECTIVE INOCULATION AGAINST A PLANT VIRUS. *Nature [London]* 131: 468.
- (25) SIMON, C. E.
1915. INFECTION AND IMMUNITY, A TEXT-BOOK OF IMMUNOLOGY AND SEROLOGY FOR STUDENTS AND PRACTITIONERS. 351 pp., illus., Philadelphia and New York.
- (26) SMITH, K. M.
1931. COMPOSITE NATURE OF CERTAIN POTATO VIRUSES OF THE MOSAIC GROUP. *Nature [London]* 127: 702.
- (27) THUNG, T. H.
1931. SMETSTOF EN PLANTENGEN BIJ ENKELE VIRUSZIEKTEN VAN DE TABAKSPLANT. *Nederland.-Indie Natuurw. Cong. Handel.* 6: 450-463, illus.

ROOT DEVELOPMENT OF PITCH PINE, WITH SOME COMPARATIVE OBSERVATIONS ON SHORTLEAF PINE¹

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INTRODUCTION

The development of the root systems of trees constitutes a relatively unexplored field of botany. Most of the older references to this subject have been based on casual observations of exposed roots along roadside cuts and eroded stream banks, or of the upturned roots of wind-thrown trees. Hence, descriptions of the root systems of trees generally have been incomplete, if not actually inaccurate.

The extensive investigations of the roots of herbaceous and shrubby plants, particularly those by Cannon (5)⁴ and Weaver (26, 27, 28), led naturally to a greater interest in the roots of trees. However, the relatively large amounts of time and labor required have tended to discourage work in this field. Problems arising in the nurseries and the relative facility with which small plants can be studied have directed research mostly toward the seedling stages. Toumey described and classified the seedling root systems of many of the more important eastern species (25) and stimulated many other investigations of a similar character.

A few studies of the roots of older trees have been carried out in this country. In general, the work done thus far has been of a preliminary character, and gives neither a complete picture of the root growth of a species from seedling to maturity on any particular site nor an adequate account of the root reactions of a species to the various sites on which it may grow. Woodroof's studies (30, 31) on the pecan constitute an exception to this statement.

Somewhat more work has been done in the north-European countries. The German work has been summarized by Büsgen and Münch (4). Perhaps the most complete study of any one species was carried out by Laitakari (17) in Finland on Scotch pine (*Pinus sylvestris* L.).

The species chosen for the present investigation was pitch pine (*Pinus rigida* Mill.), because of its wide distribution throughout the Eastern States and its remarkable tolerance of a wide range of unfavorable situations. Particularly because of this latter quality, the species promises to assume increasing importance in the forestation programs of the future, although it is not an important timber tree at present. Pitch pine is prominent among the botanically interesting flora of the pine barrens of New Jersey. The ability of the species to withstand fire evokes the wonder and admiration of all who know that unique section.

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³ Maintained at Philadelphia, Pa., in cooperation with the University of Pennsylvania.

⁴ Reference is made by number (italic) to Literature Cited, p. 1015.

Definite root studies of pitch pine have apparently never been made. Harshberger (10) made some general statements concerning the root development of the species, and published a small drawing of a tree with its root system. His observations obviously were of a most cursory character, and his figure is entirely inadequate as a portrayal of a root system. Illick and Aughanbaugh (15) mention the strong taproot of pitch pine seedlings and the fact that this root becomes relatively less significant in older trees. They measured the upturned root mass of a wind-fallen tree, recording 18 feet as the diameter of the root system of a specimen 82 feet tall. Such figures are of questionable value; probably not more than one-fourth of the actual diameter of that root system was represented in the mass torn from the earth when the tree fell.

The observations of Harshberger and of Illick and Aughanbaugh are representative of the available information on the roots of pitch pine, and of most other tree species. More precise knowledge of the root habits of trees is requisite for the judicious planning of forestation programs. In this investigation, the major objective was to study, on one site, a developmental series of root systems of pitch pine from the seedling stages to maturity. Secondary objectives included comparative observations of the root systems of normal and weak trees, of trees growing on different sites, and of pitch pine and shortleaf pine (*Pinus echinata* Mill.) growing on the same site.

FIELD WORK

DESCRIPTION OF SITE

The major part of the field work was done on the tract of the Allegheny Forest Experiment Station in the Lebanon State Forest in New Jersey. Some data were obtained from the Ockanickon area of the experiment station near Medford, N. J., and from the Mont Alto State Forest in southern Pennsylvania. Both of the locations in New Jersey are in the pine barrens, the ecology of which has been treated in some detail by Harshberger (10).

The site of most of the excavations on the Lebanon forest was an area of about 40 acres which, being traversed by a low ridge, varies from level to gently sloping. Drainage is good; excavations as deep as 9 feet did not encounter water, even on the lower ground. The soil of the ridge is typical Lakewood sand; on the lower ground it tends somewhat toward the Sassafras types. At a few places, a slightly harder, dark-colored layer was encountered below the A horizon, suggestive of the St. Johns series, but it was not well developed. These minor variations in soil did not seem to exert any appreciable direct effect on the behavior of the pine roots.

A recent paper by Lutz (20) gives detailed data on the soils of the section and a quantitative expression of their poor quality. His determinations show that the pine-barren soils are characterized by: (1) High percentage of sand; (2) low percentages of clay and total colloids; (3) low content of organic matter; (4) low contents of nitrogen and phosphorus; (5) high carbon-nitrogen ratio; (6) high acidity; (7) excessive leaching, and varying degrees of podsolization; (8) low water-holding capacity; and (9) periodic occurrences of low percentages of available moisture—the moisture content may fall below the wilting coefficient during periods of drought.

The forest cover is a mixture of white, black, and chestnut oaks (*Quercus alba* L., *Q. velutina* Lam., *Q. prinus* L.) with pitch and short-leaf pines. The oaks are mostly sprout growth averaging 4 to 5 inches in diameter breast high,⁵ and represent the growth since the last cutting for charcoal. The pines are of all sizes up to 12 inches d. b. h. Some of the larger ones are approaching, or perhaps exceed, 100 years of age. Apparently the area has not suffered severe burning for some time, as seedling and sapling pines of various sizes are fairly abundant.

The ericaceous ground cover, typical of the section, is sparse on the ridge, and composed mostly of dry-land blueberry (*Vaccinium vacillans* Kalm). Lower down on the slopes, black huckleberry (*Gaylussacia baccata* (Wang.) C. Koch) becomes predominant, with dangleberry (*G. frondosa* (L.) Torrey and Gray) appearing also on the lowest flats. A dense ground cover of the huckleberries rather effectively excludes pine seedlings. The natural inference is that saplings and older trees growing among them got started following a ground fire which had temporarily checked the huckleberries. The majority of the pines in seedling stages are now found on the higher and less densely vegetated parts of the area.

METHODS

The root systems were exposed by the dry method. The first step in the case of trees larger than sapling size was to uncover the root crown to a depth of 1 foot or more and radially about 3 feet. This operation was, of necessity, largely done by hand in "badger" fashion. With the root crown thus revealed, the most suitable position for making deeper excavations with the least destruction of roots could be determined. A hole of sufficient size to allow a man to stand in it and use a spade was then dug alongside the stump. Its ultimate depth and the size necessary to permit spading movements and prevent caving of the walls were determined, of course, by the depth of the taproot. By careful caving of the wall toward the stump, the root crown and taproot could be clearly exposed without serious mutilation. With the bases of the lateral roots thus revealed, representative ones could be selected and followed in any degree of detail desired. Exposure of the laterals also was largely a hand process, although a trowel and small hand ax were useful. An ice pick was helpful in freeing the finer roots from the soil. Excavation of vertical branches of the large laterals necessitated digging a hole in the same manner as for the taproot.

The dry method has several advantages over the alternative wet method, in which the roots are washed from the soil with a stream of water under pressure. The former can be used by an investigator working alone, it does not require proximity to a source of water or to a road by which water may be hauled, and it requires only the simplest outlay of equipment. A spade, an ax, a trowel, a hand ax, an ice pick, a ruler, and a few small pails or jars of water for saving specimen roots constitute the essentials for field work. To get complete detail of the finer root branches in sandy soils by dry excavation is not unduly difficult. The wet method is more suitable, however, for getting out seedling root systems, or parts of larger

⁵ Four and a half feet above the ground; abbreviated "d. b. h."

systems, intact. Considerable breakage and desiccation of finer roots are unavoidable by the dry method.

In this study, no attempt was made to obtain complete intact root systems except in the case of small seedlings. On larger trees the taproot and only a limited number of representative primary laterals were followed to the tips. Likewise, only representative samples of the higher orders of branching were examined in detail. Experience soon showed the general range of variation to be expected. Excavation of all the roots would have consumed more time than the results would warrant in a study of general root habit.

GENERAL FEATURES OF PITCH PINE ROOT SYSTEMS

The root system of pitch pine may be regarded as belonging to the generalized type, i. e., it is both widely spreading and deeply penetrating, but does not attain extreme development in either direction.

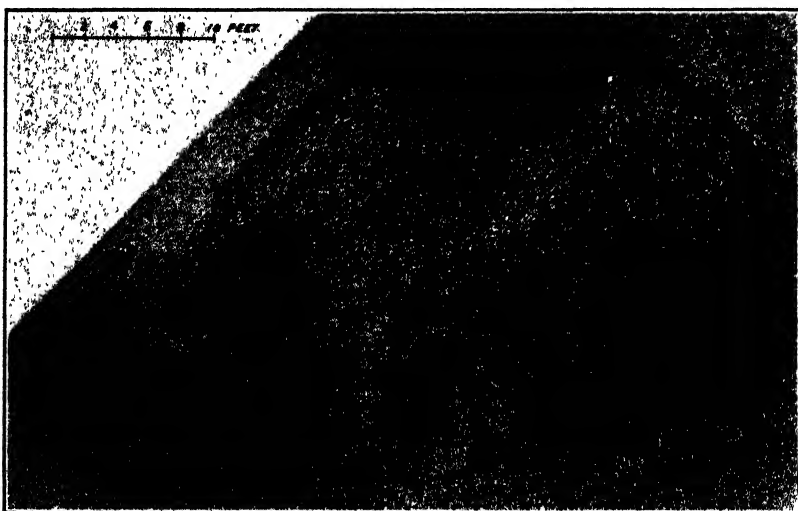


FIGURE 1.—Three-dimensional diagram illustrating the taproot and the positions of three primary laterals with their larger secondary and tertiary branches. The development of vertical roots is depicted at the right; at the left is shown a lateral of deeper origin obliquing upward; in the background is shown the horizontal development with the topsoil removed. Modeled after a tree about 30 years old.

Insofar as we have knowledge of other pines, this type of root system seems to be typical of the genus (6, 12, 17). It usually shows a definite and fairly strong taproot which frequently is found to divide, below a depth of 2 to 3 feet, into numerous branches descending at acute angles to the vertical. This feature becomes apparent only after the tree has passed the sapling stages. The major part of the lateral system originates from the root crown within 8 inches from the soil surface. These primary laterals extend radially at depths of 2 to 8 inches, rarely, if ever, turning downward appreciably. Where a dense mat of ericaceous roots and rhizomes occurs, the pine roots tend to run below it at depths of 5 to 8 inches; where the ericaceous mat is absent or poorly developed, some pine laterals will be found as close as 2 inches to the soil surface. The primary lateral system is almost entirely confined to the surface soil during the seedling and

sapling stages; later, laterals originating as deep as 2 feet may attain conspicuous size. Deeply originating laterals frequently, though not always, oblique gradually upward to the surface soil, where they proceed horizontally. The primary laterals give off both vertical and horizontal secondary branches, which rebranch commonly to the fifth and sixth orders. The term "vertical", as here used, applies only to roots growing downward; those branches which grow upward from the more deeply situated laterals soon turn in a horizontal direction and are not distinguished from those which grow horizontally from the start. The vertical branches along the basal half of a lateral may penetrate almost as deeply as does the taproot; those of more recent origin on the distal half of the lateral naturally are not so well developed (fig. 1). That the tree has contact with the subsoil under the major part of the area covered by the lateral system is a fact that is not generally appreciated and that warrants emphasis.

Many of the ultimate branches are mycorrhizal. Lateral branches of the higher orders frequently extend into the duff, where mycorrhizal development may be profuse. However, mycorrhizae are not confined to the surface soil. In the pine-barren soils, mycorrhizae seem able to develop under practically all conditions that permit growth of the pine root itself. They were observed as deep as 8 feet in drained soils and more than 3 feet below the water table in saturated soils.

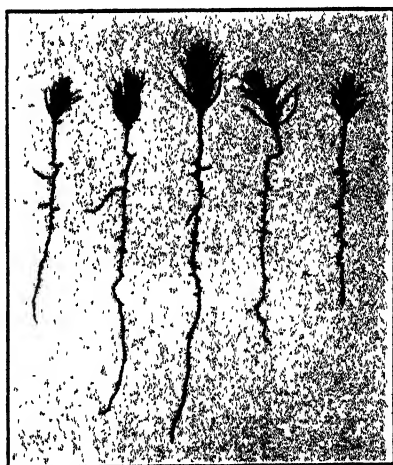


FIGURE 2.—Specimens of *Pinus rigida* seedlings taken in June from open woods. The length of the longest taproot was 5.5 inches.

ROOT DEVELOPMENT IN EARLIER STAGES OF GROWTH

SEEDLING ROOT SYSTEMS

The taproot is the most conspicuous feature of seedling root systems. Taproots of seedlings in their first year of growth varied in length from 3 inches to more than 1 foot (fig. 2). The influence on root development of local differences in site is probably more clearly seen in young seedlings than at any later stage. The maximum elongation of roots occurs on areas of clean, loose sand subjected to strong insolation. The bare sand of infrequently used roadways or trails will often yield seedlings with exceptional root development. The combined factors of shade, higher humus content of the soil, and underground competition tend to retard root elongation of seedlings growing under the tree canopy. Under these conditions, the taproots sometimes penetrate scarcely deeper than through the layers of duff and raw humus during their first year of growth. Burns (3) has shown experimentally that white pine seedlings produce the strongest root systems when grown without shade, the root system

becoming progressively weaker as shade is increased. Top development, also, was markedly retarded in the plants grown in full shade. In the relatively open woods of the pine barrens, conditions of full shade rarely occur. Hence, top development gives little indication of root development in the case of young pitch pine seedlings in nature.

By the end of the first year, recognizable lateral roots have appeared along the upper 2 inches of taproot (fig. 3). Although the strongest 2 or 3 laterals on well-developed root systems may be as long as 6 inches, most of them do not exceed 3 inches in length. Usually not more than 5 or 6 branches can definitely be identified as potential laterals on the 1-year-old seedling. At this time branching has proceeded to the second and occasionally the third order.

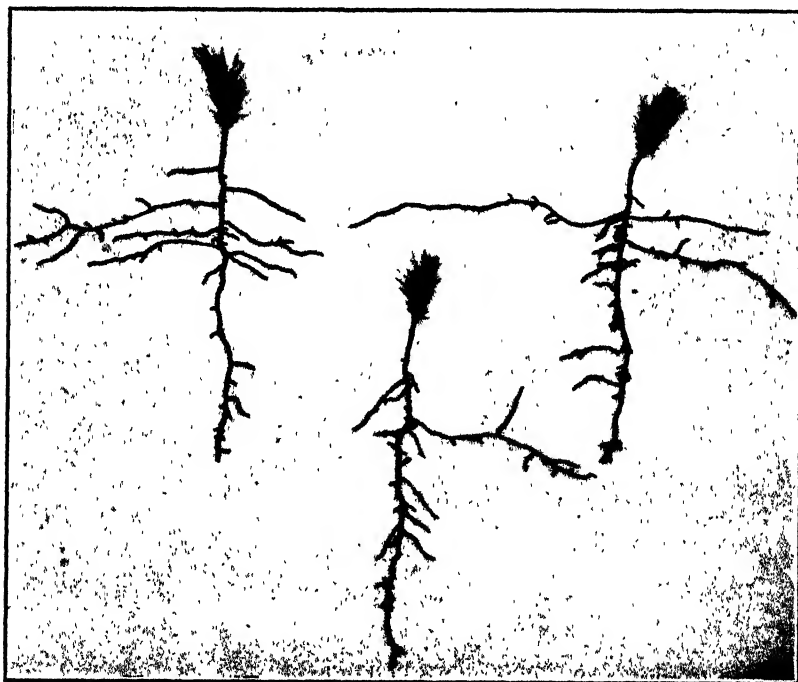


FIGURE 3.—Specimens of *Pinus rigida* seedlings taken in August from an exposed roadside. The taproots were about 8 inches long.

Mycorrhizae were already evident in June on the roots of seedlings of the current season. Such seedlings could not have been much more than 2 months old. Some apparently healthy seedlings were found on which the whole root system was sheathed in fungus mycelium. Mycorrhizae were present in abundance on 1-year-old seedlings, with some clusters showing 4 or 5 dichotomies.

The taproot may be regarded as dominating the root system up to the eighth or tenth year of the plant's life, though after the first year its dominance steadily decreases. Seedlings 4 or 5 years old, growing under conditions of moderate competition and partial shade on Lakewood sand, showed taproot penetrations of 15 to 24 inches and maximum lateral extensions of 12 to 15 inches (fig. 4). Usually there

were 6 to 8 horizontal roots exceeding 6 inches in length, the largest of which approximated the size of the taproot. Even at this early stage, the increasing importance of the lateral system is becoming apparent.

Seedlings of this age (4 or 5 years old) are typically 10 to 12 inches tall, unbranched weak, and spindling, decumbent or, at best, only feebly erect. The seedlings of both *Pinus rigida* and *P. echinata* usually topple over during the second or third year, and remain in a semiprostrate position for several seasons. By the eighth year they usually have again assumed an erect position, but a permanent crook remains at the ground line. This crook is ultimately obscured by the thickening of the stem, but frequently can still be seen in saplings 8 or 10 feet tall.

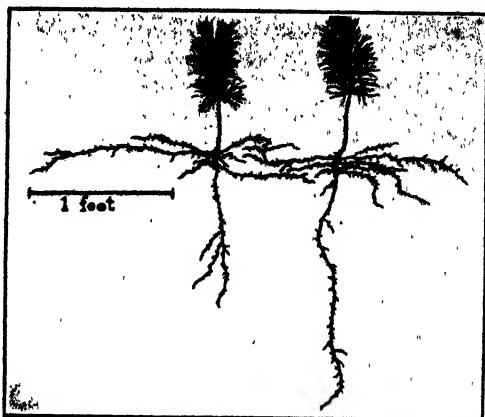


FIGURE 4.—Four-year-old seedlings of *Pinus rigida* with practically complete root systems.

Seedlings 8 to 9 years old, likewise under conditions of moderate competition and partial shade, were 1.5 to 2.5 feet tall and were beginning to branch. Though plants of this age have assumed a fairly erect position, the stems still lack rigidity. Naturally, varia-

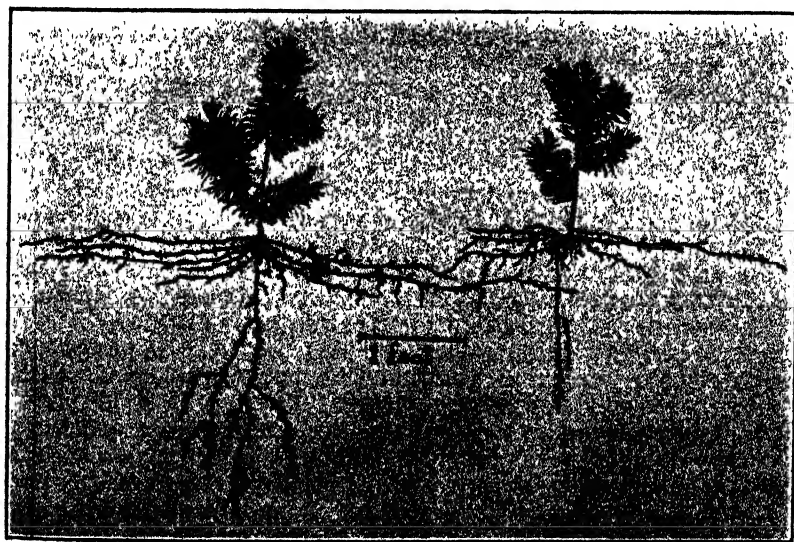


FIGURE 5.—Eight-year-old seedlings of *Pinus rigida* with practically complete root systems.

tion between individuals increases with age, both above and below ground. Taproot penetrations varying from 21 to 34 inches were found on sites apparently very similar (fig. 5). The stronger laterals approximated 2 feet in length, though one was found which measured

40 inches. The number of identifiable laterals averaged 10 or more. Some of the stronger ones bore secondary branches almost 1 foot long. Branching had proceeded commonly to the third and occasionally to the fourth order, exclusive of mycorrhizae.

Some comparison was made with seedlings of *Pinus echinata* of the same age and on the same sites. The development of the two species was very similar; no differences were noted which did not readily fall within the limits of variation within each species. It is the writer's opinion that detailed analysis of many specimens will be necessary to determine any differences which may exist between the seedling root systems of the two species. However, it was noted that the stems of seedlings of *P. echinata* tend to stand more rigidly erect than do those of *P. rigida*.

ROOT SYSTEMS OF SAPLINGS AND SMALL TREES

By the eleventh or twelfth year the pitch pine seedling has acquired a degree of rigidity heretofore lacking, the growth rate has increased, giving an aspect of vigor, and annual whorls of branches are being regularly developed. The young plant at this age begins to appear like a tree. This acceleration of growth undoubtedly is coordinated with root development; it could not take place until an adequate absorbing system had been formed and sufficient contact made with the subsoil to insure a constant water supply. The dimensions attained by the roots indicate an even more striking acceleration of growth underground than that displayed by the tops. The eighth to tenth years mark the inception of a period of rapid root elongation. This period also witnesses the appearance of secondary branches of conspicuous size.

It should be borne in mind that, although it is convenient to speak of these younger stages in terms of years, age probably is relatively insignificant. The development of the plant is more properly to be viewed as an orderly process, the speed of which is governed, within limits, by the environmental conditions. Thus, a stage of development ascribed to 10-year-old trees in New Jersey might be reached several years earlier, or later, under other conditions.

A fairly typical sapling, 11 or 12 years of age, was 4 feet tall, and 0.75 inch in diameter at the ground line. The current season's leader was 8.5 inches long, and that of the past season, 7 inches. The tap-root reached a depth of 4 feet, the longest lateral root reached out 8 feet, and 3 other laterals exceeded 4 feet in length. Ten major laterals had originated within 6 inches from the surface of the soil, and 5 more were present at greater depths. The longest lateral bore 12 secondary branches ranging from 1 to 2.5 feet in length. The strongest secondary branch descended vertically from one of the other primary laterals to a depth of 3.5 feet.

The appearance of greater numbers of branches, both primary and secondary, as the seedling grows older does not mean that new branches have originated at points remote from the tip of the parent root. As will be discussed later, branch roots normally originate only in tip regions before the advent of secondary thickening. Many branches never become more than a few inches long, and eventually die and disintegrate. Certain ones, however, which at first are undistinguishable from the ephemerals, later undergo increased growth in both length and thickness and become recognizable as permanent structures. These account for the apparent increase in the number of major branches.

The root dimensions given indicate the relative decline of the taproot and the mounting prominence of the system of primary lateral branches. The taproot, of course, retains its importance as an organ of central anchorage, and as the mechanical and physiological center of the root system. Its upper part is the conduit through which all liquids must pass. Its function as an absorbing organ, however, becomes more and more insignificant as the tree develops. Vertical secondary branches, or sinkers, contact the subsoil and thus relieve the taproot of its earlier vital role—insurance against death by drought. Ultimately, the taproot, including its descending branches, may not greatly exceed the stronger sinkers with respect to the amount of absorbing area.

A somewhat larger sapling illustrates further the relative decline of the taproot. It also illustrates a fact borne out by observations on larger trees, that beyond the seedling stages the size of the top furnishes but little indication of the depth or size of the taproot. The lateral system, however, is fairly closely correlated with stem development. After a little experience, the observer can predict the general dimensions of the lateral system. This sapling was 14 years old by ring count, 7.5 feet tall, and 1.5 inches in diameter at the ground line. The taproot reached a depth of only 3 feet. It gave off 20 laterals, 14 of which originated at depths not greater than 6 inches. The longest one measured 10.5 feet, 3 others exceeded 8 feet in length, while the remainder ranged from 2 to 8 feet. Primary laterals shorter than 2 feet were not considered. The 2 strongest laterals each bore about 30 secondary branches approaching or exceeding 1 foot in length. Very few were more than 3 feet long. At least 3 vertical secondaries reached into the fourth foot of soil, thus exceeding the taproot in depth. Branching had proceeded to the sixth order, exclusive of mycorrhizae.

In the consideration of secondary branches, only those which approached or exceeded 1 foot in length were recorded numerically. Smaller branches were numerous, but irregularly distributed. Many were dead, or apparently dormant, and destined eventually to slough off. Their importance as absorbing organs is, at present, purely speculative, but would seem to be negligible on the older parts of the root system. A branch which has attained a length of 1 foot is growing, or previously has grown, more vigorously. Its basal parts have undergone some secondary thickening, and the epidermis has been replaced by the characteristic flaky bark, giving the aspect of a permanent organ. A reasonably close correlation exists between the basal diameters and the lengths of pine roots. Thus, their lengths can be estimated without complete exposure. Branching to the higher orders is considerably more profuse on lateral branches than on vertical ones.

Primary laterals less than 5 feet long rarely have many such secondary branches. The latter appear in increasing numbers as the primary elongates. By the time a primary has become 10 feet long, it may have 20 to 40 secondaries 1 to 4 feet long. Usually, one-half to three-fourths of these are horizontal, and the remaining one-fourth to one-half are vertical. Such a primary lateral thus has developed from a root into a root system in itself. Its zone of absorption extends down to the moister sands of the subsoil, and several feet laterally on either side in the topsoil. Subsequent growth of the pri-

mary lateral is simply the extension, vertically, laterally, and linearly, of this zone of root occupancy. However, downward growth does not keep equal pace with the lateral and linear advance. Hence, the zone of root occupancy of a primary lateral undergoes a gradual relative flattening as development progresses, and a constantly increasing proportion of the root system as a whole becomes localized in the surface soil. The data on larger trees illustrate this tendency.

A specimen tree, 14 feet tall, 2.5 inches d. b. h., and about 17 years old, may be cited. This individual had originated as a sprout, and therefore probably was slightly larger for its age than trees developing directly from seed. Suitable specimens of seedling origin of this general size were almost entirely lacking in the locality. The taproot was rather atypical. It divided about 9 inches below the surface of the soil, the two roots subsequently uniting in a natural graft, and then redividing. One branch ended at a depth of 3 feet. The other, after forking twice more, reached a depth of slightly more than 5 feet. Twenty-two primary laterals had originated at depths of less than 1 foot, and several small ones were found at deeper levels. Four laterals exceeded 15 feet in length, the longest being 19 feet. A fifth one measured 12 feet; the others were of various shorter lengths. The number of secondary branches varied from 40 to 75 on the 4 largest laterals. Horizontal secondaries as long as 6 feet were found, at which size tertiary branches of conspicuous size were present. None of the vertical secondaries, or sinkers, reached deeper than 5 feet.

The same general sequence of development is followed by the secondary branches as by primaries. Their early growth is largely manifested in elongation, most of the side branches being small, delicate, and ephemeral. When a length of 4 or 5 feet has been attained, permanent branches of the next higher order first become conspicuous. The same sequence may be repeated again by tertiary branches on still older root systems.

Branches of the second or higher orders differ from the primary laterals in certain growth habits. The former frequently are found to oblique upward into the raw humus of the soil surface, whereas the latter usually run at slightly deeper levels. Also, branches of the higher orders often branch so extensively near the end that the parent root can no longer be identified. It is doubtful whether such a root can subsequently undergo any pronounced elongation. Laitakari (17) observed a similar situation in Scotch pine, and states that very little further elongation occurs. Primary laterals, on the other hand, always maintain their identity in a morphologically distinct tip capable of indefinite growth.

A specimen tree about 30 years old, 22.5 feet tall, and 3.5 inches d. b. h. may be cited to illustrate a more advanced stage of development. This tree grew on a rather high, sterile site, whereas the preceding one was located on lower, but nevertheless well-drained, ground. Though such minor variations in site do not seem to be reflected noticeably in root development, they cannot be wholly ignored in comparing the two trees. Excessive drainage on the higher site quite probably stimulated deeper penetration of the vertical roots.

The taproot of this tree reached a depth of 9 feet. Its upper part was strongly developed, being 5 inches in diameter 6 inches below the soil surface and 3 inches in diameter at a depth of 2 feet. At that level

it divided into 3 descending branches; these subsequently divided further. At the 5-foot level there were 15 of these descending branches distributed within a circular area about 2 feet in diameter (fig. 6). Most of them did not extend deeper than 7 feet, but the two longest ones reached depths of 8.5 and 9 feet. Growing tips were fairly abundant, and active mycorrhizae were collected as deep as 7 feet.

There were 20 sizable laterals, 13 of which originated at depths of less than 6 inches; the other 7 were found at greater depths down to the 2-foot level. The longest lateral measured 31 feet, another 29 feet, and at least 3 others exceeded 20 feet in length. The smaller ones included in the count were estimated to be about 8 feet long.

A few, of still smaller size, were not recorded. Secondary branches were not extensively excavated. One of the larger secondary laterals was 9 feet long, another was exposed for 11 feet without recovering the tip; several others were estimated to be of equal, or greater, length. One sinker was observed to end about 5.5 feet deep, but others probably reached deeper. The largest sinkers generally may be expected to approximate the taproot in depth.

On any tree secondary branches that can be classified as large in proportion to all secondaries are relatively few. The majority of them are comparatively small. Some data taken from the last-mentioned specimen tree illustrate this point. The secondaries were tabulated in three size categories: (1) Length greater than 5 feet, (2) length 2 to 5 feet, and (3) length less than 2 feet. In most cases, the length was only estimated from the features of the basal part of the root, but as noted above, such estimations can be sufficiently accurate for the purpose. Of 348 secondary branches tabulated from 7 primaries, 22 were classed in group 1, 104 in group 2, and 222 in group 3. The most richly branched primary bore 95 secondaries, of which 6 were classed in group 1, 31 in group 2, and 58 in group 3. The smaller primaries usually had no secondaries falling in group 1.



FIGURE 6.—Taproot exposure of a 30-year-old tree. The excavation was 4.5 feet deep when the photograph was taken; it was subsequently extended to a depth of 9 feet to recover the deepest branches.

GENERAL CONCLUSIONS FROM STUDY OF ROOT SYSTEMS IN EARLY STAGES

The plan of this paper thus far has been to present general morphological data from the developmental point of view. The growth of the root system has been followed from the seedling stages to the conditions found in immature trees 20 to 25 feet tall. Subsequent root development, as illustrated by larger trees, proceeds along somewhat different lines. Hence, certain generalizations applicable to the earlier growth stages are now presented.

PROPORTIONS OF VERTICAL AND HORIZONTAL SECONDARY BRANCHES

A summation of data with respect to the proportions of vertical and horizontal secondary branches is given in table 1. Since the horizontal category includes branches on both lateral sides of the parent root, it is evident that the primary puts out branches in three general directions, and that the sinkers represent approximately one-third of the total. This three-way distribution of branches cannot be correlated with the anatomy of the protosteles. Although the primary xylem is not infrequently triarch in the roots of pitch pine, the diarch condition is most common.

INCREASING PROMINENCE OF THE LATERAL SYSTEM

Mention has been made above of the increasing prominence of the lateral system of roots as the tree develops. In the pine barrens, the taproot usually is the longest and most conspicuous root up to the eighth to tenth years of the plant's life. Soon after that time, the strongest laterals linearly outstrip the taproot, and eventually nearly all of them exceed the taproot in length. Mention also has been made of the tendency of the root system to flatten out and become relatively more localized in the topsoil as development progresses. This change comes about largely through the more rapid growth of the primary laterals as compared with that of the taproot and sinkers. A second, and less important, factor is the tendency of the secondary laterals to surpass the sinkers in growth rate during later development. This is evident only in trees 10 or more feet tall, and after the secondaries have attained a length of 4 or 5 feet.

TABLE 1.—The proportions of vertical and horizontal secondary branches of three pitch pines

Tree no.	Primary roots tabulated	Secondary roots				
		Total	Vertical		Horizontal	
	Number	Number	Number	Percent	Number	Percent
1 ¹	3	80	37	46	43	54
2 ²	9	286	96	34	190	66
3 ³	6	284	75	26	209	74

¹ An unusually vigorous 10-year-old sapling.

² 17-year-old tree cited in text.

³ 30-year-old tree cited in text.

Table 2 illustrates these trends of development. The increasing size of the figures from top to bottom in the sixth and seventh columns

is most informative. The larger ratios shown by the last two figures in the ninth column are also undoubtedly significant; the other figures in that column probably only express variations around a norm of 1.0.

TABLE 2.—Data illustrating the increasing prominence of the lateral system and the relative flattening of the entire system as the tree develops

Height of tree	Age of tree	Depth of taproot	Depth of strongest sinkers	Length of longest primary lateral	Primary laterals longer than taproot	Ratio of longest primary lateral to taproot	Length of longest secondary laterals	Ratio of secondary laterals to sinkers
<i>Inches</i>	<i>Months</i>	<i>Inches</i>	<i>Feet</i>	<i>Inches</i>	<i>Number</i>		<i>Feet</i>	
1.5	2-3	5		1.5		0.3		
	<i>Years</i>							
3.0	1	8		3.0		.4		
11	4	18		15		.8		
22	8	27		33	(¹)	1.2		
<i>Feet</i>		<i>Feet</i>		<i>Feet</i>				
2.7	10	3.3	1	5.5	3	1.7	1	1.0
4	12	4	3.5	8	5	2.0	2.5	.7
4.5	11	2.5	3	12	13	4.8	4	1.3
7	10	3	3	14	12	4.7	2.5	.8
7.5	14	3	4	10.5	12	3.5	3.5	.9
14	17	5	5	19	12	3.8	6	1.2
22.5	30	9	7	31	17	3.4	12	1.7

¹ No data.

RELATION OF LATERAL SPREAD OF ROOTS TO HEIGHT OF TREE

A simple criterion for estimating the area occupied by the root system would be of some practical value. However, the limitations of such a criterion must be recognized. Because of inherent variations, and variations due to the environmental complex, close estimations of root development are impossible when based only on above-ground characters. In the pine barrens, about four of the laterals may be expected to exceed the trunk of the tree in length. The longest lateral usually is approximately 1.5 times as long as the stem. This applies only to trees less than 25 feet tall and to trees that have not been forced to spindle upward. Data illustrating this generalization are given in table 3.

It is even more difficult to estimate the depth of taproots. The figures in the first and third columns of table 2 indicate that the stem and taproot attain linear equality somewhere between 2 and 4 feet. Previously, the taproot was longer; after the interval of equivalence, the stem is always longer. Nine feet appears to be near the maximum depth attained by pitch pine taproots in the locality.

TABLE 3.—Data showing the relation of the maximum lateral spread of roots to height of tree

Height of tree	Laterals exceeding the stem in length	Length of longest lateral	Ratio of longest lateral to height of tree	Height of tree	Laterals exceeding the stem in length	Length of longest lateral	Ratio of longest lateral to height of tree
<i>Inches</i>	<i>Number</i>	<i>Inches</i>		<i>Feet</i>	<i>Number</i>	<i>Feet</i>	
11		13	1.2	2.5	4	6.3	2.3
11		15	1.4	4.0	4	8.0	2.0
18		26	1.4	4.5	7	12.0	2.7
18		27	1.5	7.5	2	9.0	1.2
20		40	2.0	7.0	3	14.0	2.0
				7.5	4	10.5	1.4
<i>Feet</i>		<i>Feet</i>		14.0	4	19.0	1.4
2.5	4	4.7	1.9	22.5	6	31.0	1.4
2.8	5	5.3	1.9				

RELATION BETWEEN ROOT DIAMETER AND LENGTH

Little has been said in the preceding discussion concerning the diameters of roots. From a physiological standpoint, diameters are of far less significance than lengths of roots and volume of soil occupied. Basal diameters, particularly those of horizontal roots, are roughly proportional to lengths, and can be used to some advantage in estimating the lengths of laterals. In cases where there is appreciable enlargement at the point of origin, measurement should be taken beyond the region of pronounced tapering. For a general estimate of the length of a root, the basal diameter is measured in millimeters; the length expectancy is an equivalent number of feet. For example, a root of 5 mm in diameter at the base is about 5 feet long; a root 30 mm in diameter at the base is about 30 feet long. Naturally, there are rather wide variations, with a somewhat greater tendency of lengths to fall short of, rather than to exceed, the expect-

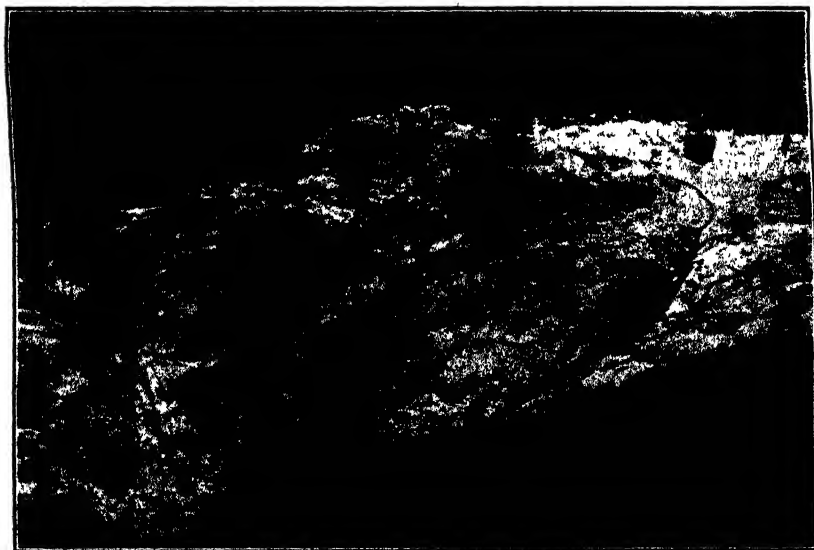


FIGURE 7.—Exposure of part of the root system of a 17-year-old pitch pine showing the ropelike character of the laterals. The tools mark the ends of roots. The root on the right was 19 feet long.

tancy according to this simple formula. The length is generally inversely proportional to the degree of branching, i. e., the parent root is shorter when well branched than when poorly branched. The diameters in millimeters and the lengths in feet were tabulated for 53 primary laterals from seven trees. The ratio of the length figure to the diameter figure was calculated for each root. The average of these ratios was 0.85. This expresses the tendency, noted above, of lengths to fall slightly short of the simple ratio of 1.0.

It can be seen that these lateral roots are rather slender structures. Since branches usually are distinctly smaller than the parent root, and on older roots are relatively sparsely distributed, the larger laterals may aptly be described as "ropelike" in appearance (fig. 7).

During the younger stages sinker roots maintain the same proportions as laterals, but after they reach a depth of 3 or 4 feet, elongation

is retarded. Growth in diameter may continue, resulting in greater thickness in relation to length. The diameter of a taproot furnishes no reliable indication of its length.

ROOT DEVELOPMENT OF TREES APPROACHING MATURITY

As has been mentioned, root development proceeds along somewhat different lines as the tree approaches maturity. The size at which this change occurs may be designated, more or less arbitrarily, as follows: Height, 25 feet; diameter breast high, 4 inches; radial spread of roots, 30 to 35 feet; and age, 30 years. Early root development involves a rather steady increase in length, both laterally and vertically, during which the ropelike character of the laterals is maintained without marked thickening at any point and general correlations exist between extent of root systems and size of tops and between diameters and lengths of roots. Subsequent growth adds little or nothing to the radial spread of the root system. Büsgen and Münch (4), in a footnote, indicate that a similar sequence of development has been observed in Europe (probably on *Pinus sylvestris*). Instead of further elongation, a marked thickening becomes apparent along the basal 1 to 3 feet of the stronger primary laterals. The smaller primaries, and to some extent, the secondary laterals, continue to elongate, thus increasing the density of roots within the 30-foot radius. Ultimately, the originally smaller primaries attain lengths approximately as great as those of the strongest laterals; they then cease elongating and undergo basal thickening. Further penetration of the subsoil by the taproot and the strong sinkers near the root crown practically ceases, downward growth being confined almost entirely to branches of those roots and to the younger, more distal sinkers.

The taproot of pitch pine, which is nearly always a conspicuous feature of younger root systems, becomes relatively insignificant on some older trees. In such cases, the function of anchorage is taken over by several of the innermost sinkers, which have become much thickened (fig. 8). The size of these sinkers varies inversely with the size of the taproot.



FIGURE 8.—The central roots of a mature pitch pine, showing the strong development of sinkers associated with a weak taproot. The longer roots reached a depth of about 8 feet. The tree was 48 feet tall, 9 inches d. b. h., and 85 years old.

The basal thickening of the primaries very frequently is more pronounced in the vertical plane, resulting in "planklike" roots. The asymmetry rarely extends outward more than 3 feet. Undoubtedly, it is a mechanically efficient means of bracing the tree against the increased stresses of a larger crown. Rigg and Harrar (22, p. 396) noted an extreme development of this tendency in the shallow root systems of trees growing in sphagnum peat, and, apropos of the mechanical efficiency of such modifications, state that "the strength of beams of varying transverse sectional shapes approximates the square of the vertical dimension when the horizontal dimension remains the same."

These planklike roots frequently are decidedly eccentric; that is, the morphological center of the root is below the actual center, as a

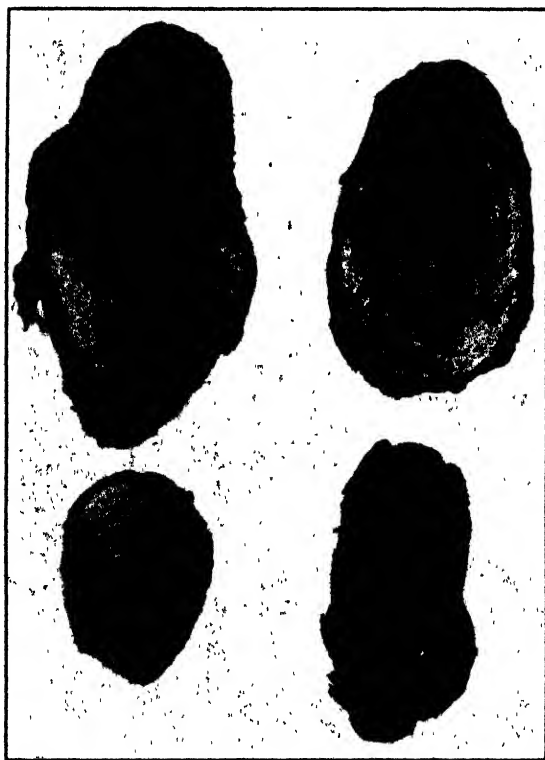


FIGURE 9.—Cross-sectional views of four lateral roots cut close to the taproot. All show narrowing in the vertical plane, and the two lower ones show eccentric thickening on the upper side.

result of greater thickening on the top than on the under side (fig. 9). Thus, shallowly situated laterals often grow out of the ground near the stem base, producing the familiar buttressed effect. Although many deciduous trees display this feature more markedly, it shows plainly in pitch pine and probably is a wide-spread habit. Laitakari (17) mentions it in discussing the roots of *Pinus sylvestris*. Vertically narrowed roots and buttresses attain striking dimensions in some tropical trees. Obviously, buttresses are mechanically efficient structures; the same amount of material would give greater support in that position than in the strictly horizontal position of the original root. Investigations of tropical trees indicate, however, that buttresses cannot always be explained as a simple response to mechanical stress. They may develop when lateral stresses are negligible. Air and water relations appear to be influential factors (7, 9). The formation of the characteristic buttresses of the bald cypress (*Taxodium distichum* (L.) Rich.) has been shown to be dependent on a combination of water plus air (16).

The buttresslike development of lateral roots of mature, erect trees suggests that the roots function mechanically as props or braces,

and that the thickening develops in direct response to the increasing stresses produced by the growing crown. Undoubtedly, supporting roots also function mechanically as tensile guy wires, but that role is minor and is secondary to the propping action. Laitakari (17) has pointed out the tendency of Scotch pine to produce more and stronger laterals on the side opposite that of the prevailing winds, i. e., in the propping position. To check this tendency further in pitch pine, leaning trees were examined. The one-sided stress on the roots of such individuals is distinct and constant. Of 4 such trees examined, 3 showed a very marked concentration of laterals on the side under the inclined trunk, with no sizable surface laterals on the opposite side (fig. 10). This indicates clearly that those roots in the propping position are thickened in response to stress; those in the guy-wire

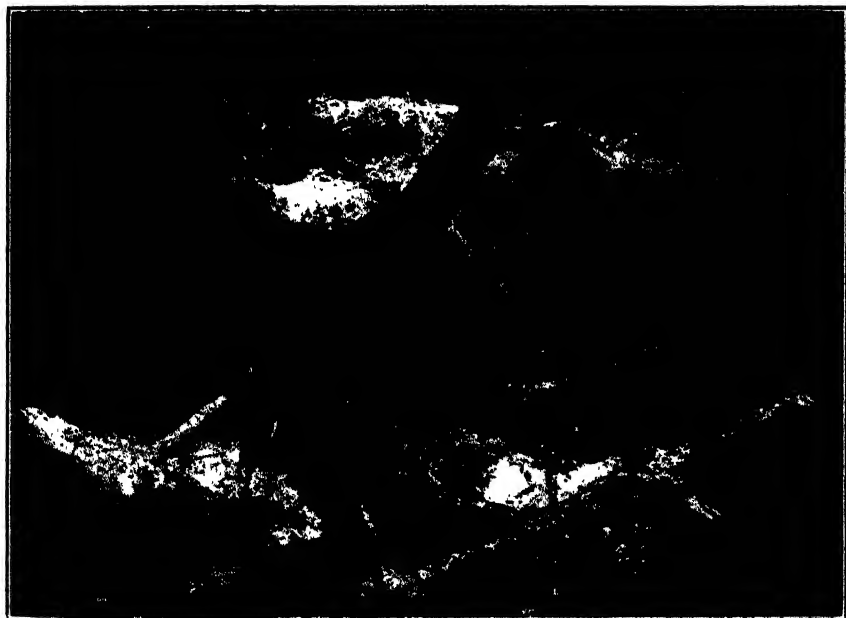


FIGURE 10.- Exposed roots of a leaning tree, showing the majority of the lateral roots in the propping position.

position do not thicken, but may possibly break under the strain, die, and disappear.

Examination of the roots of the fourth leaning specimen, in which there was no correlation between lean and root development, did not weaken this conclusion. It was a sprout, only slightly inclined and relatively small (2 inches d. b. h. and 15 feet tall). The symmetrically arranged root system had been laid down by the parent tree; the sprout had not yet become old enough or heavy enough to alter its symmetry appreciably.

The positive correlation between the growth of roots and of tops has been pointed out by numerous investigators. It is practically regarded as axiomatic by most students of plant roots. The subject was not given particular attention in this investigation. However, the leaning trees discussed above were all rather weak, slow-growing

specimens. The number and size of the primary laterals and the thickness of the taproot were distinctly inferior in comparison with those of normal trees of the same size. In view of the open character of the forest, it is doubtful that the weakness of the specimens was due to suppression by larger trees. It seems most probable that both the leaning and the general weakness of the tops had resulted from, or were coincident with, an originally weak root system.

It seems quite probable that the processes of maturing and senescence are correlated with the tree's inability to extend the root system into new territory. It is obvious that the demands for support placed upon the inner root system increase enormously as the top attains mature stature. The material requirements for strengthening near the root crown may be an important factor in precluding further root extensions. In any event, a fertile field for speculation is offered by the question of whether maturing and senescence are wholly internal and protoplasmic, with the cessation of root extension as a result, or whether the mechanical difficulties of support and of transportation through an ever-widening root system are contributory causes of those processes.

ORIGIN OF ROOT BRANCHES

The endogenous origin of the branches of roots is one of the fundamental facts of plant anatomy. Normally, branches emerge along the maturation zone of the root tip, and along the tender parts farther back which have not undergone marked secondary thickening. The completion of a solid ring of secondary wood generally precludes further branching. On an actively growing root, this probably takes place during the second year; on roots growing less vigorously, either of two types of behavior may occur: (1) Secondary thickening may be indefinitely delayed, and the period of possible branching equally prolonged; (2) secondary thickening may advance to within 5 mm of the tip, resulting in thick, blunt-ended roots with a barely perceptible tip of tender tissue. The former is usually associated with dormant lateral roots; the latter condition is most often found in vertical roots deep in the subsoil.

The growing tips of the major extending roots are thick and succulent, about 3 mm in diameter. The tips of branches at emergence usually are distinctly smaller. In cases where the parent tip has died (a surprisingly common occurrence), a replacement tip emerges which, from the start, has the attributes of the parent root. It resembles the latter in size and vigor of growth, and at once assumes a forward rather than a lateral direction (fig. 11). Sometimes two such tips emerge opposite each other; the immediate result, while the dead parent tip persists, is an apparent trifurcation; ultimately the dead root disintegrates, leaving a bifurcate condition.

The formation of replacement tips was not observed to take place when dying-back had extended into the region of complete secondary thickening. In that event, an existent branch may undergo greater than average growth and after the disintegration of the dead parts appear to be the normal continuation of the parent root. This is nearly always what has happened in those occasional cases in which a major lateral root appears to become vertical, or vice versa. The abrupt change of direction marks the origin of a branch which assumed dominance after the death of the parent root beyond that point.

The roots of many herbaceous plants, which do not undergo extensive thickening, may produce branches at any point. Moisture frequently stimulates abundant branch production on such roots in regions far removed from the growing tip. The fact that the woody root, in some species at least, is only slightly or not at all capable of such response warrants considerable emphasis, particularly in respect to the taproot. With such species, of which pitch pine appears to be one, it means that the original complement of primary branches on the seedling taproot never can be increased in later life. It is highly probable that an originally poorly branched taproot, or one deprived of its branches in any way, will result in a weak root system and predispose toward general weakness of the tree. The best cultural care is, to some extent, wasted on a tree with an inadequate root system. Of course, when the number is reduced, roots may become somewhat larger and branch more profusely than usual, but it is doubtful that such development can be fully compensatory in respect to absorbing area. Certainly, reduction in the number of primary laterals weakens the support of the tree and predisposes toward wind throw in later life.

The above statements do not apply with equal force to all tree species. Adventitious roots, arising both from stems and from other roots, are mentioned occasionally in the literature. Luncz (19), in reviewing some European work, cites Austrian pine and cherry as examples of trees whose roots may give rise to adventitious branches, and states that such branches are not morphologically distinguishable from the original roots. If adventitious roots ever arise on either pitch or shortleaf pines, they must require a more powerful stimulus than normally occurs in nature. Examination of scores of roots showed that branches of all sizes, on both species, universally extended inward to the protoxylem of the parent root, and therefore had originated when that region was young (fig. 12). The only possible exception found was in cases where the roots had been severed smoothly with a sharp instrument. Some small branches had subsequently arisen exogenously near the cut ends. Examples of this were found at the scene of stump-grubbing operations of the preceding winter. No observations were made of exogenous branches of greater age.

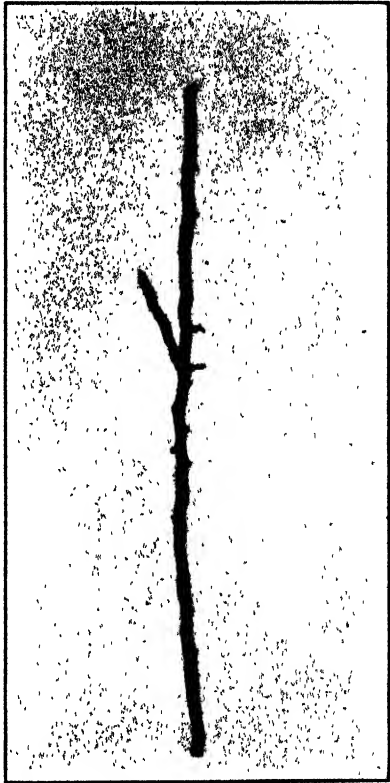


FIGURE 11.—A young replacement tip developing after the death of the terminal portion of the parent root. Note its relative size and direction of growth in comparison with the remnants of normal side branches.

GROWTH OF ROOTS UNDER WATER

Probably the most striking single feature of pitch pine roots observed in the course of this investigation is the fact that they develop extensively below the water table in saturated soils. So far as the writer is aware, no similar observation on a mesophytic tree

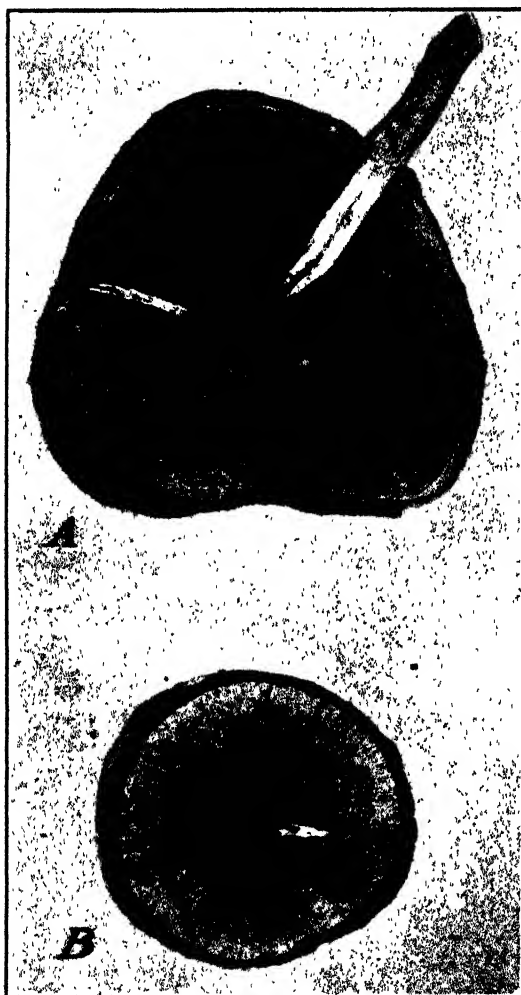


FIGURE 12.—Cross-sectional views of roots showing the central origin of branches: A, A section from a taproot, 5.5 inches in diameter; B, section from a very small primary lateral, shown about natural size.

has been reported. Such hydric species as *Taxodium distichum* undoubtedly root under water, but in practically all descriptions of the roots of mesic trees, and even of such bog inhabitants as American larch and black spruce, they are reported to develop entirely above the saturated zone. Textbooks generally state that root penetration ceases when the water table is encountered (4, 24), and much has been written of the deleterious effects of poor drainage on various economic plants. Rigg and Harrar (22) found that none of the six conifers growing in the bogs of Washington extended roots below water. According to Woodroof (30), the roots of the pecan cannot tolerate submergence. The longleaf pine (*Pinus palustris* Mill.), which in some respects is the southern ecological equivalent of pitch pine, is reported by Heyward (12) not to penetrate below the water table on poorly drained sites. However, Hesselman (11) notes the occurrence of vigorously growing spruce stands in Sweden in the vicinity of springs, even where the water level comes to the surface. Although no data are given on root development, the trees obviously do produce roots under water in such situations. Adamson (1) describes an Indian tree (*Terminalia arjuna* Bedd.) which inhabits

has been reported. Such hydric species as *Taxodium distichum* undoubtedly root under water, but in practically all descriptions of the roots of mesic trees, and even of such bog inhabitants as American larch and black spruce, they are reported to develop entirely above the saturated zone. Textbooks generally state that root penetration ceases when the water table is encountered (4, 24), and much has been written of the deleterious effects of poor drainage on various economic plants. Rigg and Harrar (22) found that none of the six conifers growing in the bogs of Washington extended roots below water. According to Woodroof (30), the roots of the pecan cannot tolerate submergence. The longleaf pine (*Pinus palustris* Mill.), which in some respects is the southern ecological equivalent of pitch pine, is reported by Heyward (12) not to penetrate below the water table on poorly drained sites. However, Hesselman (11) notes the

river banks and extends roots out into the saturated soil of the river bed.

The root behavior of white cedar (*Chamaecyparis thyoides* (L.) Britton, Sterns, and Poggenberg), which is the typical bog tree of southern New Jersey, has not been investigated, but in view of the sites supporting it, it probably does root below the water level. Pitch pine, however, is most at home on higher, well-drained sites, and intermingles only slightly with the white cedar in the narrow dividing ecotone. The pine can live among the cedars only by overtopping them; once the cedar canopy closes, any pine beneath it is doomed. The occasional pine that towers among the cedars points clearly to the fact that it is not the site itself, but the competition, that generally excludes them.

Pitch pine is to be regarded as a member of that wide-spread plant fraternity which, demanding little except a place in the sun, must find its place as a rule in the left-over, unfavorable areas that will not support the "nobler" species. Such are the sterile, fire-scorched sands of the Coastal Plain, or the wind-swept ridges of the mountains, both of which are typical pitch pine sites. It invades burned-over areas, abandoned fields, and clearings, where individuals may persist, but where, if conditions are favorable, reproduction soon becomes impossible in competition with other species. Although its tolerance of competition is low, its tolerance of a wide range of site factors is remarkable. Sandy soils or clay, fertile or infertile, drained or saturated, situations xeric or mesic or hydric—all are acceptable to this indiscriminating species.

Three trees were examined with respect to root growth below the water table.⁶ The largest one was 33 feet tall and 5 inches d. b. h. It was located on a steep incline that bounded the valley of a creek. The water table was about 3 feet below the surface at the spot where the tree stood. Lateral roots extended up the incline into typical Lakewood sand; on the lower side, they extended into the flat valley bottom where water was only about 6 inches below the surface, with sphagnum moss and cinnamon fern (*Osmunda cinnamomea* L.) to testify to the saturated condition. The laterals were of the usual type, and put down sinkers regardless of the proximity to water (fig. 13, A). They were aligned generally parallel with the incline of the soil surface, both uphill and downhill. The taproot penetrated the zone of saturation as a strong shaft, 4 inches in diameter at the level of submergence. It broke up into a fan-shaped mass of branches along its fifth foot. This fan, which was about 2 feet wide and 4 to 6 inches thick, was a most peculiar tangle of roots of various sizes, unlike anything found on drained soils (fig. 13, B). As a mass, it ended at a depth of 6.5 feet, though a few individual roots reached slightly deeper. Many of the tips were alive and growing slowly.

A large proportion of the ultimate fine branches were mycorrhizal,⁷ a condition which was quite unexpected. The literature contains

⁶ The method of securing the central root systems from saturated soils was as follows: The topsoil was removed around the root crown down to the water, and the lateral roots were severed. A strong pole was chained to the stump and operated as a lever over a fulcrum of logs. With this, 3 or 4 men could pull stumps of the sizes described. Fractionally every root was secured intact, as the sand was held enmeshed in the root tangle and was lifted as a solid mass.

⁷ The true mycorrhizal character of these roots has been verified by K. D. Doak, of the Allegheny Forest Experiment Station.

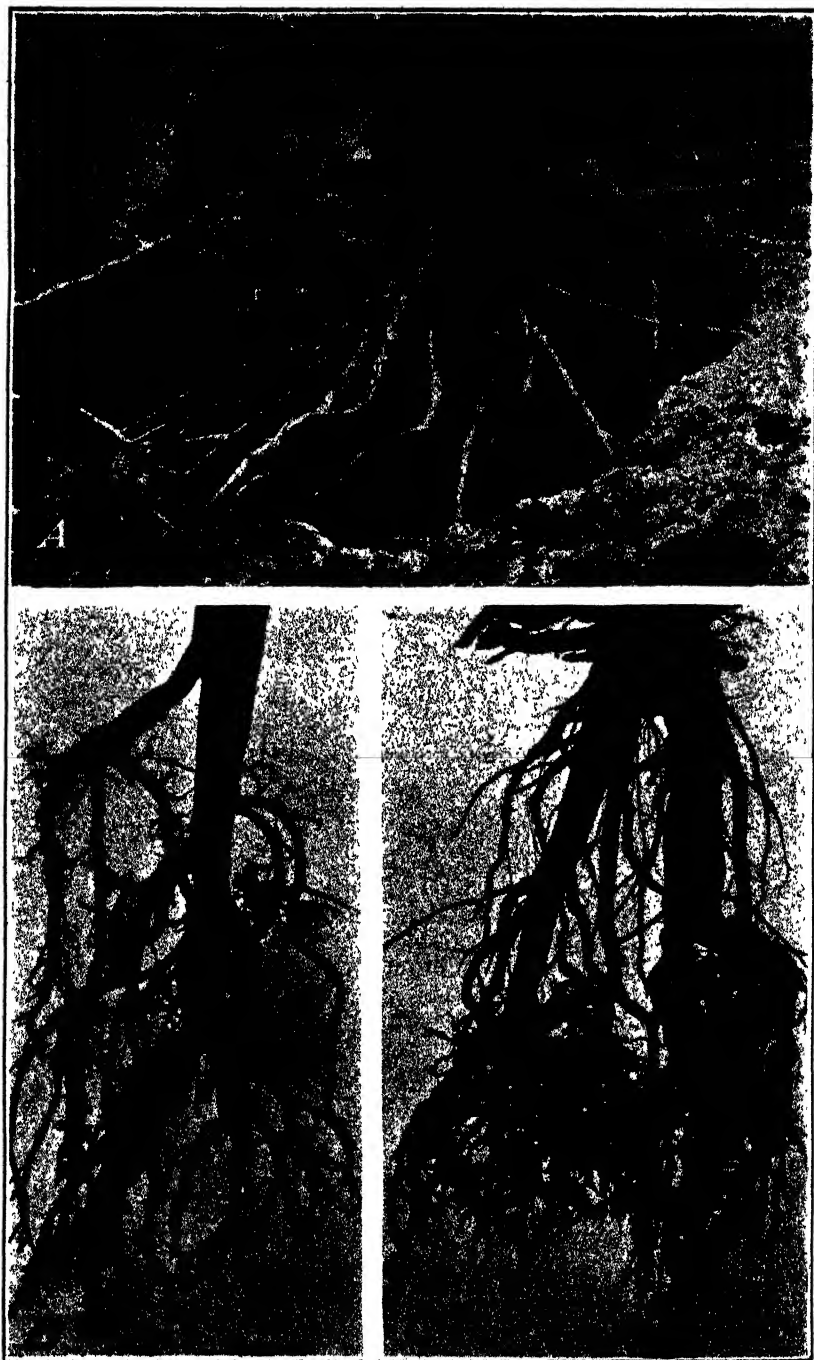


FIGURE 13.—Central roots of trees from saturated soil: *A*, Preliminary excavation of the largest tree, with water standing in the bottom of the hole. *B*, Under-water portion of the taproot of the same tree. It reached a depth of 6.5 feet. *C*, Roots of a smaller tree, from a below the soil surface; these roots reached a depth of 5 feet.

very few definite statements with respect to the occurrence of mycorrhizae in nature on submerged roots. The negative findings of Bondois (2) constitute the only direct observations on this topic known to the writer. The fact that mycorrhizae had not been reported on submerged roots and that good drainage seems essential for the successful synthesis of mycorrhizae in culture (21) has led to the general opinion that they do not develop under saturated conditions. In some respects the presence of mycorrhizae far below a permanent water table is even more remarkable than the growth of the pine roots themselves. Interesting questions are raised as to the identity and physiology of the fungus involved—questions to which there are at present no answers.

The other two trees taken from saturated soils grew on a lower site, in what probably was originally the ecotone between white cedar and pine stands. (Clearing had disturbed the natural vegetation.) The water table was about 8 inches below the surface in dry weather; for several weeks after a rainy period it remained only about 4 inches down. The site was not a true bog as known in glaciated regions. The surface soil to a depth of 6 to 10 inches was composed largely of fibrous plant debris, hummocks of cinnamon fern, and sphagnum moss. It was densely interwoven with living roots and rhizomes. This fibrous mat rested on sand, with a sharp line of demarcation.

The specimen trees were 4 inches d. b. h. and about 20 feet tall, somewhat smaller than the one discussed above. Both were vigorous and healthy in appearance, the more recent internodes being 12 to 18 inches long. One of these trees was essentially taprooted, though the shaft forked into two almost equal and parallel parts and divided extensively into a fan-shaped mass of many-branched and contorted roots (fig. 13, C). It ended at a depth of 4 feet, with a few individual roots going about 1 foot deeper. The finding of two such fans indicates that their formation is a normal response to the saturated condition. Why this is the case must, at present, be left to conjecture. The branches did not originate wholly on opposite sides of the taproot, which would seem to eliminate diarchy of the protostele as an explanation. In fact, many of the roots were triarch. This tree, also, showed numerous growing tips and mycorrhizal clusters.

The second smaller tree had no taproot, but was anchored by four strong sinkers located a few inches out from the stump. They ended at depths of 2.5 to 3 feet.* One of these obliques downward at an angle of about 45°, which is notable as the only example found on any tree of a major root following such a course. (Major roots are almost always either definitely vertical or definitely horizontal.) This tree had developed no fanlike structure.

These three trees demonstrate that pitch pine can thrive on saturated soils; that it will root solidly and deeply on such soils, with no more than the usual danger of windthrow; and that mycorrhizae can develop under conditions of saturation.

Before applying these conclusions generally, it will be necessary to examine trees in other regions and on other soil types. It is possible that conditions in the pine barrens permit a greater degree of aeration of ground water than usually occurs elsewhere. As is well known, many herbaceous plants that are intolerant of saturation will grow in water culture if properly aerated. Hole (14) has shown that some trees, at least, respond in the same manner. Hesselman (11) attributes the growth of spruces in the saturated soils near springs to the

unusual aeration of the water. Further investigation is necessary to determine whether it is the species or the site that is most unique in southern New Jersey.

Plant roots growing under water often show anatomical differences, particularly in the development of air spaces. Adamson (1) reports the presence of lacunae in both the primary and secondary cortex of the submerged roots of *Terminalia arjuna*. However, no air spaces, nor marked looseness of the cortical cells, can be discerned in the submerged roots of pitch pine. Hesselman (11) mentions that neither pine nor spruce roots develop special air passages when submerged. Bondoio, in a paper dealing with anatomical features of the roots of mesic trees grown in water (2), noted that spruce (the only conifer studied) did not develop cortical air spaces, though this did occur in several hardwood species.

ROOT DEVELOPMENT ON HEAVIER SOILS

Conditions did not permit extensive comparative studies of *Pinus rigida* on widely different soil types, though such studies are much to be desired. Some observations and partial excavations of root systems were made near Mont Alto, Pa., where pitch pine grows on the higher ridges in the mountains. The soils are raw, podsolized, stony, and high in clay. They are rendered so sticky and compact by the clay that spading is almost impossible without preliminary loosening with a pick. These soils offer far greater physical resistance to root penetration than do the sands of the Coastal Plain and in all probability retard root elongation irrespective of other factors.

Two small trees, 11 and 14 feet tall, were examined. The following differences, in comparison with New Jersey specimens, were noted:

(1) Vertical roots were generally weaker in both thickness and length. Taproots of these trees reached depths of 3 to 3.5 feet.

(2) Primary laterals were shorter, the longest ones scarcely exceeding the stem in length.

(3) Laterals frequently ran closer to the soil surface than in the New Jersey sands. This probably is a result of the greater moisture-retaining capacity of the clayey soil.

(4) Laterals followed more tortuous courses through the soil owing to the resistance of the soil itself and to obstructing rocks.

(5) Secondary branches were generally more numerous per linear unit of primary root.

(6) Primary laterals had undergone much more basal thickening than is usual on New Jersey trees of the same size. This is undoubtedly correlated with the weakness of the taproots and the consequently greater burden of support placed upon the laterals. Stronger prevailing winds, also, may be a factor.

These generalizations, though derived from a limited number of observations, are in essential agreement with statements in the literature relating to tree roots. Hence they carry more weight than could be granted to them if they were not thus supported. Of particular interest are the observations of Rigg and Harrar (22) on the roots of conifers growing in sphagnum peat. They found markedly greater root elongation in this loose substratum than in more compact and resistant soils. They agree with Büsgen and Münch (4) that mechanical obstructions rather than conditions of nutrition retard root elongation.

ROOT FUSIONS

Frequent references are made in the literature to root fusions, both within the same system and between different systems. The occasional persistence of life in coniferous stumps is usually explained as resulting from intersystemic fusions with the roots of a living tree (fig. 14). In this investigation, such natural roots grafts were found only rarely, and with one exception involved root of the same tree. Some were found on both pitch and short-leaf pines. Close aggregation of trees is most conducive to root grafts. The fact that such situations were avoided in choosing specimen trees probably accounts for the small number observed.

ABSORBING AREA
AND THE PROBLEM
OF ABSORPTION

This report is concerned primarily with the gross features of root systems. The description and interpretation of the ultimate branching patterns and of functional areas of absorption entail more detailed study, involving tip-by-tip analyses and laboratory experimentation.

In a general way, the terminal parts of vertical roots are coarse and sparsely branched (fig. 15); those of horizontal roots are more slender and much more profusely branched. On vertical roots, the diarchy or triarchy of the protostele often can be determined readily by the alinement of the branches (fig. 16); on horizontal roots, the alinements usually are obscured by the profusion and contortion of the branches. Roots feeding in the surface humus are most intricately branched; the network often is so complex that it is next to impossible to obtain any sizable portion intact and free from organic debris. The intricacy is enhanced further by innumerable mycorrhizal clusters. Though mycorrhizae are found at all levels (fig. 15), they occur in greatest abundance in the surface organic layers of soil.



FIGURE 14.—A natural root fusion on *Pinus echinata*.

The amount of functional absorbing area is, in the final analysis, one of the most significant features of root systems. The extent and degree of branching of the skeletal framework of a root system is important only insofar as it brings the absorbing membranes of young tips into contact with the necessary water and nutrients. Exact knowledge of the root parts wherein absorption occurs is very meager. It is not known with certainty how much of the terminal region of a root actively absorbs, or whether dormant tips can absorb, or whether mycorrhizae are important as absorbing organs. According to the usual concept, absorption takes place primarily in the zone of extension found between the apical meristem and the suberized mature region of a growing root. Scott (23) has reiterated this general idea,



FIGURE 15.—Tip regions of two vertical roots taken 7 feet deep in drained soil. The clusters are mycorrhizae. Note the paucity of permanent branches.

and pointed out that, with retarded growth due to drought or other unfavorable conditions, suberization processes continue and encroach on the absorbing regions of the root. The tip ultimately may be completely enclosed by a suberized covering.

It has been observed by various investigators, and verified by the writer in respect to the species studied, that the roots of pines do most of their growing in the spring and fall. Finding a growing tip was a rare occurrence in July, August, or early September. The dormant tips found generally during that period were brown and shrunken as compared with growing ones. Free-hand sections showed the cortical cells to be in a flaccid condition, and the cell walls were brown inward to the endodermis. They appeared to represent thoroughly that state described by Scott wherein the root tip is completely enclosed by a suberized covering. If it is admitted that active absorption takes place only in growing tips, and if Scott's statement is accepted that

"the distribution of suberization may be used as an indication of the delimitation or curtailment of the absorbing region of the root", then the incongruous situation is presented of absorbing area being reduced to a minimum when transpiration stresses are greatest. Scott supplies no answer to this question. Perhaps that actually is the case, and the tree obtains its water during the late summer months solely through the few vertical roots whose tips are not completely dormant. Though many of the tips found deep in the subsoil also are dormant, and the number of growing ones seems wholly inadequate to supply the needs of the tree, the fact remains that the tree passes through the dry summer period annually without visible deleterious effects. The prevailing paucity of root hairs on pitch pine roots renders the mode of water entry even more uncertain. The whole problem of the location and mechanics of water absorption in the pine offers a fertile field for further research.

SIGNIFICANCE OF THE ROOT HABITS OF PITCH PINE

The relation between initial root habit and the ability of seedlings to maintain themselves on various sites has been emphasized by Toumey (25) and is generally recognized by foresters and ecologists. To establish itself on a xeric site, the seedling must promptly make contact with the more permanent reservoir of moisture in the subsoil. Those species which push their outposts farthest into the mid-

western prairie are notably deep-rooted. *Quercus macrocarpa* Michx. and *Juglans nigra* L. may extend their taproots down more than 4 feet during the first season of growth (13). Species with inflexibly shallow roots are restricted to sites where the topsoil is always moist.

The excessively drained sands of the pine barrens constitute a relatively xeric situation; hence the native pitch pine might be expected to put down a vigorous taproot during the first season. As noted in the discussion of seedling root systems, 1-year-old plants reach a depth of 1 foot or more on exposed situations. Although this is not a striking development, it does bring the roots into contact with sands wherein the water content only very rarely is depleted to the point of the wilting coefficient. In comparing this growth with that of xeric broad-leaved trees of the Middle West, it must be borne in mind that the latter undoubtedly transpire much more water than

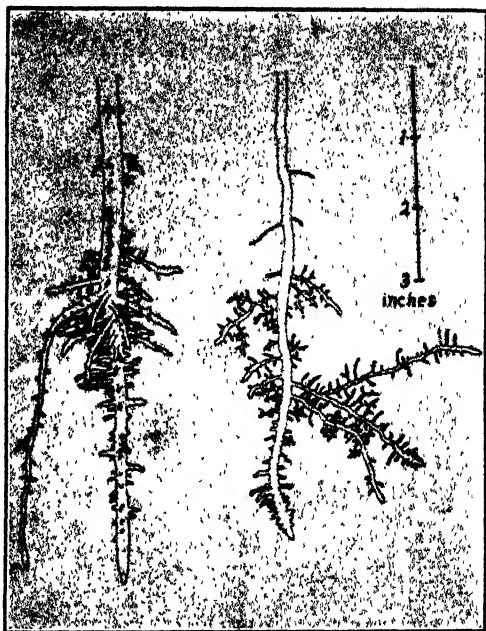


FIGURE 16.—Line drawing of two tips of vertical roots. The one on the left was triarch and that on the right, diarch.

do needle-leaved seedlings in the more humid atmosphere of the eastern seaboard and therefore require a deeper and more extensive root system.

The literature indicates that seedlings of pines, in general, develop deeper root systems than most other native gymnosperms, but, at best, fall far short of the growth attained by such hardwoods as black walnut and the more xeric oaks and hickories. *Pinus palustris*, growing in the southern sandy Coastal Plain, ordinarily does not penetrate the soil deeper than 1 foot during the first season (18). *P. ponderosa* Laws., the dry-land tree of the Rocky Mountain area, does not develop unusually deep seedling root systems (8). Evidently the successful ecesis of pines on dry areas cannot be wholly ascribed to root adaptations. Other features, including reduced transpiration, undoubtedly are significant. Pitch pine is equaled or surpassed, with respect to depth of seedling root systems, by several other pines and by various hardwoods. From an ecological viewpoint its seedling root habit is only one of the complex of characters by means of which the plant is adapted to xeric situations. Though it would be presumptuous to account for the prominence of the species in the flora of the pine barrens on the basis of any one character, resistance to fire probably is most decisive (20).

With respect to the extent of root systems of mature trees, pitch pine conforms generally with other species for which information is available. The similarity between the root systems of pitch and shortleaf pines has been noted above. The root development of jack pine (*Pinus banksiana* Lamb.) evidently closely parallels that of *P. rigida* and *P. echinata* (6). Longleaf pine and Scotch pine, at least under certain conditions, may considerably exceed pitch pine in spread of roots. Lengths of 70 to 80 feet are reported (12, 17). Laterals of the bur oak attain lengths of more than 60 feet (29). The horizontal roots of the pecan may extend laterally 30 feet on 12-year-old trees, and generally maintain lengths of about twice the lateral spread of the crown (30, 31). Such figures, of course, are not strictly comparable, since the various species grew under widely differing conditions of soil and climate. They show, however, that despite soil conditions generally conducive to root elongation the extent of root systems of pitch pine is not exceptional. Furthermore, they show the fallacy of the old notion that the spread of root systems of trees approximates the spread of the crowns. In trees beyond the sapling stages, the spread of roots is nearly always at least twice, and in many cases four or more times that of the tops. Equivalence of spread above and below ground is to be regarded as the exception rather than the rule.

Depth of roots is more generally responsive to soil conditions than is their lateral spread. The figures reported for pitch pine in this paper certainly do not approach the maximum depths attainable by tree roots. Even on the conservative basis of a depth of 8 feet and a lateral spread of 30 feet, the root system of a tree is seen to occupy an enormous volume of soil. Trees in nature nearly always are spaced so closely that considerable intermingling of root systems results. With these are associated the roots of all the subdominant shrubby and herbaceous plants of the forest floor. In general, the underground parts of the plants of the forest are more intimately

associated with each other than are the tops. Particularly on dry or infertile soils, underground relations probably determine the density of the vegetation. When viewed with a full appreciation of the extent and interrelations of root systems, it may justifiably be contended that the complexity of the forest community attains its greatest expression underground.

From a silvicultural standpoint, several suggestions are warranted on the basis of this study. They may be listed as follows:

(1) Only seedlings with strong, well-branched root systems should be planted. A root system poorly branched in early life will always be weak, both as a supporting and as an absorbing structure, and will result in a weak tree.

(2) Pitch pine is not recommended for planting in shallow soils overlying solid rock. The tendency to develop fairly deep vertical roots is strongly inherent in the species. Complete inhibition of the vertical development would result in weakly anchored trees, and probably would cause physiological disturbances.

(3) In the sandy soils of New Jersey, pitch pine may be planted on sites where the water table is as close as 8 inches to the soil surface. Development of vertical roots under such conditions is not markedly inhibited, and the trees are firmly anchored. Planting on sites where the water table remains at the soil surface is not recommended without further study. Aeration of the surface soil where the majority of the lateral roots are found may be essential to the health of the tree. Also, planting on heavy soils with a high water table is not recommended until the reaction of the species on such areas has been investigated.

(4) Pitch pine probably can best be used in mixture with other species. The natural oak-pine mixtures characteristic of the pine barrens constitute a pertinent suggestion. All observations indicate that the greatest productivity of the soil is to be realized by following this hint from nature. Pitch pine roots extensively, but not intensively. Some intermingling of the roots of adjoining trees may take place without initiating marked competition between them. In the case of such intolerant species as pitch pine, crown closure does not necessarily indicate complete closure underground. The mixed planting, in which the intolerant pine is given a start over more tolerant associated species, thus promises the greatest return.

COMPARATIVE OBSERVATIONS ON *PINUS ECHINATA*

Shortleaf pine (*Pinus echinata*) is an important component of the forest on drained soils of the Lebanon area. It occurs in mixture with *P. rigida* and the oaks, or, locally, in almost pure stands. However, it does not follow the pitch pine into low areas of poor drainage. Presumably, it cannot tolerate a high water table. In the Lebanon area, shortleaf pine appears to be even more intolerant of reduced light than pitch pine. The trees are remarkably well self-pruned, and if at all crowded tend to become spindling and weak.

Although this investigation was centered around pitch pine, observations sufficient to establish certain similarities and differences between the two species were made. It has been pointed out that no constant differences are apparent in the younger stages of growth

(up to 10 years of age). Differences are apparent in the roots of trees approaching maturity, but the stage of development at which those differences become conspicuous was not determined. The most striking difference is in the development of the taproot. *Pinus echinata* produces a much more powerful and massive central shaft (fig. 17) that maintains its thickness to greater depths and displays less tendency to divide into an array of descending branches. The depths attained by the vertical roots of the two species are essentially the same.

The root systems are similar in general form and extent. The same general relationships between spread of roots and height of



FIGURE 17.—Taproot of an 85-year-old shortleaf pine, showing its massive character.

tops, and between lengths and diameters of laterals, seem to prevail as in pitch pine. Also, the growth of laterals follows the same sequence, i. e., elongation and the maintenance of a ropelike character during the first decades of the tree's life, followed by retarded elongation and the initiation of basal thickening as the stresses incident to an enlarging crown increase. Thickening, however, is less pronounced in shortleaf pine, inasmuch as the taproot has assumed the greater burden of support, and for the same reason strong supporting sinkers near the root crown are few or wanting.

These comparative generalizations concerning *Pinus echinata* are based on examinations of three specimen trees. Two of them were somewhat weak and spindling, 25 feet and 31 feet tall, respectively, and 4 inches d. b. h.; the third was a mature tree, 8.5 inches in diameter, 45 feet tall, and about 85 years old. It may be noted, incidentally, that the longest

root excavated during the entire investigation was found on the somewhat atypical 25-foot specimen of *P. echinata*. The taproot of this tree gave off only 2 sizable laterals, the larger of which was 10 by 5.5 cm in cross section at the base, and 50 feet long. The root was unusually well branched; obviously, this was somewhat compensatory for the paucity of primary laterals. It is very doubtful, however, that the 2 primary laterals, even though exceptionally well developed, could equal the usual complement of 15 to 30 primaries in absorbing area. Certainly such a root system is mechanically weak as a structure for anchorage and support.

The suggestions given for silvicultural management of pitch pine hold equally well for shortleaf pine, except that this species cannot tolerate situations where the roots reach saturated soil.

SUMMARY

The root system of *Pinus rigida* was studied by the direct, dry method in the pine-barren section of New Jersey. It is to be classed in the generalized type. It attains a moderately extensive development both vertically and horizontally. The basic plan consists of a taproot from which 15 to 30 horizontal branches originate and extend radially in the surface layers of soil. From these primary branches horizontal and vertical secondary branches develop; these in turn give off tertiary branches, and so on. Normally, the root system occupies a roughly circular area of topsoil; in all of this area except the marginal parts it has extensive contacts with the subsoil through the development of vertical, or sinker, roots.

Seedlings are prominently taprooted. They reach depths varying from 3 to 12 inches during the first season of growth.

The taproots of seedlings 4 to 5 years old reach depths of 15 to 24 inches, and the strongest laterals approximate the same length. Plants of this age are semidecumbent; a permanent crook persists at the ground line long after they have become rigidly erect.

The taproots of plants 8 to 9 years old reach depths of 1.5 to 2.5 feet, and the strongest laterals approximate the same length. Plants of this age usually are erect.

Saplings 12 years of age are beginning to display regular whorls of stem branches, and are assuming the aspect of a tree. Taproots reach depths of 3 to 4 feet, and the strongest laterals extend 6 to 8 feet. The period between the eighth and twelfth years of the young tree's life witnesses the following developments:

- (1) An acceleration in the growth rate of primary lateral branches, as a result of which the radial lateral spread of the root system becomes approximately twice the length of the taproot, and 1.5 to 3 times the length of the stem. Hence, the lateral roots displace the taproot as the most prominent feature of the root system.

- (2) Retardation of the growth rate of the taproot as it penetrates the subsoil below the 3-foot level.

- (3) The attainment of linear equivalence of stem and taproot, prior to which the taproot was longer, and after which the stem is always longer. The difference in length between stem and taproot constantly increases with the growth of the tree.

- (4) The attainment of conspicuous size by secondary and tertiary branches on the stronger primary laterals.

Root growth between the ages of 12 and 30 years, or, on the basis of stature, between the heights of 4 and 25 feet, is characterized as follows:

- (1) Continuous elongation of the primary laterals until lengths of 25 to 35 feet are attained.

- (2) Maintenance of a ratio of about 1.5 to 1 between the length of the strongest laterals and the height of the tree.

- (3) Maintenance of a ratio approximating 1 mm to 1 foot between basal diameters and lengths of lateral roots.

- (4) Continuous elongation of branches of the higher orders, increasing the density of roots within the occupied volume of soil.

(5) Continuous elongation of vertical roots, which, however, after reaching depths of 3 to 4 feet, grow much more slowly than the surface laterals, and practically cease to grow at depths of 8 to 9 feet.

As a result of the processes listed above, a constantly increasing proportion of the root system as a whole becomes localized in the surface soil.

After trees have reached a height of about 25 feet, and a diameter breast high of about 4 inches, root development enters gradually upon a different phase. It is characterized as follows:

(1) Cessation of elongation by the stronger primary laterals and by the taproot and stronger sinkers.

(2) Marked thickening of the basal 2 or 3 feet of the stronger primary laterals. The increment usually is greater in the vertical plane, forming narrowed, planklike roots; in many cases, the increment is added almost entirely on the top side, resulting in eccentrically thickened, buttresslike supporting roots. This basal thickening increases the mechanical strength of the central root system, and in all probability is initiated by the stresses incident to an enlarging crown.

(3) Continued elongation of the smaller primary laterals until they approximate the length of the stronger ones, after which they, too, undergo basal thickening.

(4) Some continued growth of branches of the higher orders, resulting in increasing density of roots in both the topsoil and the subsoil.

(5) A tendency toward marked thickening of the innermost sinkers. Such thickening occurs in response to the requirements of the tree for support, hence the development is inversely proportional to the strength of the taproot.

Lateral roots function mechanically as props or braces, and only incidentally as guy wires. Thickening is stimulated by compression, and not by tensile strain. This is conclusively demonstrated by the eccentric development of the root systems of leaning trees.

Poor development of tops is associated with inferior root systems.

Root branches, irrespective of size, can be traced inward to the primary xylem of the parent root, thus indicating that they originated when that region of the parent root was young. Hence, a root system poorly branched in youth will always be poorly branched, and will predispose toward weakness of the tree. Adventitious root branches appear to be absent in pitch pine, except that in rare cases they may develop in wound tissue following clean severance of a root.

Replacement tips develop following the death of the terminal portion of a root only when death has not extended back into the region where secondary wood completely encircles the primary xylem.

Pitch pine is capable of extensive root growth below the water table in saturated soils. The descending branches of taproots under water sometimes are arranged in a peculiar and characteristic fan-shaped mass, the explanation of which is obscure. Trees growing on saturated soils appear to be in full health and vigor.

On sloping sites, the primary lateral branches generally parallel the soil surface, both uphill and downhill.

On heavier soils, root development tends to be less extensive, both horizontally and vertically, than it is in the sandy soils of the Coastal Plain.

Root fusions occur occasionally, on both *Pinus rigida* and *P. echinata*, between roots of the same or of different systems.

The fine roots of the higher orders, through which most absorption presumably occurs, are most profusely developed in the upper soil layers, and frequently extend into the raw surface humus. The ultimate branches of vertical roots are generally coarser and are produced in much less profusion. Nearly all root tips become dormant, and many die, during the dry periods of midsummer. Growth takes place mostly in the spring and fall.

Mycorrhizae are a conspicuous feature of pitch pine roots. Their period of growth coincides with that of nonmycorrhized tips. They are found at all depths on both drained and saturated soils, but attain their greatest profusion in the surface organic layers.

With respect to adaptations to xeric sites, the root systems of pitch pine show no apparent superiority over those of many other species of trees.

Certain suggestions are offered with respect to silvicultural practices.

The root development of *Pinus echinata* in New Jersey is fundamentally similar to that of *P. rigida*. The most marked difference is the tendency of *P. echinata* to develop a much stronger and more massive taproot, with which is correlated a weaker development of secondary supporting roots. This species does not invade areas where the water table is high.

LITERATURE CITED

- (1) ADAMSON, R. S.
1910. NOTE ON THE ROOTS OF TERMINALIA ARJUNA, BEDD. New Phytol. 9: 150-156, illus.
- (2) BONDOIS, G.
1913. CONTRIBUTION A L'ETUDE DE L'INFLUENCE DU MILIEU AQUATIQUE SUR LES RACINES DES ARBRES. Ann. Sci. Nat., Bot. (9) 18: 1-24, illus.
- (3) BURNS, G. P.
1914. STUDIES IN TOLERANCE OF NEW ENGLAND FOREST TREES. I. DEVELOPMENT OF WHITE PINE SEEDLINGS IN NURSERY BEDS. Vt. Agr. Expt. Sta. Bull. 178, pp. 127-144, illus.
- (4) BÜSGEN, M., and MÜNCH, E.
1929. THE STRUCTURE AND LIFE OF FOREST TREES. Transl. from German by T. Thomson. Ed. 3, rev. and enl., 436 pp., illus. New York.
- (5) CANNON, W. A.
1911. THE ROOT HABITS OF DESERT PLANTS. 96 pp., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 131.)
- (6) CHEYNEY, E. G.
1932. THE ROOTS OF A JACK PINE TREE. Jour. Forestry 30: 929-932, illus.
- (7) FRANCIS, W. D.
1931. THE BUTTRESSES OF RAIN-FOREST TREES. Kew Bull. Misc. Inform. 1931 (App. 1): 24-26, illus.
- (8) HAASIS, F. W.
1921. RELATIONS BETWEEN SOIL TYPE AND ROOT FORM OF WESTERN YELLOW PINE SEEDLINGS. Ecology 2: 292-303, illus.
- (9) HABERLANDT, G.
1914. PHYSIOLOGICAL PLANT ANATOMY. Transl. from 4th German ed. by M. Drummond. . . . 777 pp., illus. London.
- (10) HARSHBERGER, J. W.
1916. THE VEGETATION OF THE NEW JERSEY PINE-BARRENS; AN ECOLOGIC INVESTIGATION. 329 pp., illus. Philadelphia.
- (11) HESSELMAN, H.
1910. OM VATTNETS STRECKHÅLT OCH DESS INVERKAN PÅ SKOGSMARKENS FÖRSLUMPNING OCH SKOGENS VÄXTLIGHET. Meddel. Statens Skogsförälsksanst. [Sweden] 7: [91]-125, illus. [In Swedish. Résumé in German, pp. [xiii]-xvi.]

- (12) HEYWARD, F.
1933. THE ROOT SYSTEM OF LONGLEAF PINE ON THE DEEP SANDS OF WESTERN FLORIDA. *Ecology* 14: 136-148, illus.
- (13) HOLCH, A. E.
1931. DEVELOPMENT OF ROOTS AND SHOOTS OF CERTAIN DECIDUOUS TREE SEEDLINGS IN DIFFERENT FOREST SITES. *Ecology* 12: 259-298 illus.
- (14) HOLE, R. S.
1918. RECENT INVESTIGATIONS ON SOIL-AERATION. PART II. WITH SPECIAL REFERENCE TO FORESTRY. *Agr. Jour. India* 13: 430-440, illus.
- (15) ILLICK, J. S., and AUGHANBAUGH, J. E.
1930. PITCH PINE IN PENNSYLVANIA. Pa. Dept. Forests and Waters Research Bull. 2, 108 pp., illus.
- (16) KURZ, H., and DEMAREE, D.
1934. CYPRESS BUTTRESSES AND KNEES IN RELATION TO WATER AND AIR. *Ecology* 15: 36-41, illus.
- (17) LAITAKARI, E.
1927. MÄNNYN JUURISTO MORFOLOGINEN TUTKIMUS. THE ROOT SYSTEM OF PINE (*PINUS SILVESTRIS*); A MORPHOLOGICAL INVESTIGATION. *Acta Forest. Fennica* 33: 1-380, illus. [In Finnish. Summary in English, pp. [307]-380.]
- (18) LENHART, D. Y.
1934. INITIAL ROOT DEVELOPMENT OF LONGLEAF PINE. *Jour. Forestry* 32: 459-461.
- (19) LUNCZ, G.
1931. RECENT RESEARCH WORK ON THE ROOT SYSTEMS OF FOREST TREES. *Internatl. Rev. Agr.* 22: 239-243.
- (20) LUTZ, H. J.
1934. ECOLOGICAL RELATIONS IN THE PITCH PINE PLAINS OF SOUTHERN NEW JERSEY. *Yale Univ. School Forestry Bull.* 38, 80 pp., illus.
- (21) MCARDLE, R. E.
1932. THE RELATION OF MYCORRHIZAE TO CONIFER SEEDLINGS. *Jour. Agr. Research* 44: 287-316, illus.
- (22) RIGG, G. B., and HARRAR, E. S.
1931. THE ROOT SYSTEMS OF TREES GROWING IN SPHAGNUM. *Amer. Jour. Bot.* 18: 391-397, illus.
- (23) SCOTT, L. I.
1928. THE ROOT AS AN ABSORBING ORGAN. II. THE DELIMITATION OF THE ABSORBING ZONE. *New Phytol.* 27: 141-174, illus.
- (24) TOUMEY, J. W.
1928. FOUNDATIONS OF SILVICULTURE UPON AN ECOLOGICAL BASIS. v. 1, illus. New York and London.
- (25) ———
1929. INITIAL ROOT HABIT OF AMERICAN TREES AND ITS BEARING ON REGENERATION. 4th Internatl. Cong. Plant. Sci. Proc. 1: 713-728, illus.
- (26) WEAVER, J. E.
1919. THE ECOLOGICAL RELATIONS OF ROOTS. 128 pp., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 286.)
- (27) ———
1926. ROOT DEVELOPMENT OF FIELD CROPS. 291 pp., illus. New York.
- (28) ——— and BRUNER, W. E.
1927. ROOT DEVELOPMENT OF VEGETABLE CROPS. 351 pp., illus. New York.
- (29) ——— and KRAMER, J.
1932. ROOT SYSTEM OF *QUERCUS MACROCARPA* IN RELATION TO THE INVASION OF PRAIRIE. *Bot. Gaz.* 94: 51-85, illus.
- (30) WOODROOF, J. G.
1933. RELATION OF THE ROOT SYSTEM OF PECAN TREES TO NURSERY AND ORCHARD PRACTICES. *Ga. Expt. Sta. Bull.* 176, 15 pp., illus.
- (31) ——— and WOODROOF, N. C.
1934. PECAN ROOT GROWTH AND DEVELOPMENT. *Jour. Agr. Research* 49: 511-530, illus.

THE IDENTIFICATION OF CERTAIN VIRUSES AFFECTING LEGUMINOUS PLANTS¹

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INTRODUCTION

There are numerous reports in the literature concerning the transmission of legume mosaic viruses to various hosts. The failure, however, to describe adequately the viruses and the diseases which they cause has resulted in much confusion. The overlapping in the host range of certain viruses makes it impossible in many instances to identify a virus from a brief description of symptoms or a mere report of successful transmission from one host to another. In a previous paper (17)³ it was shown that several distinct viruses may infect the common bean, *Phaseolus vulgaris* L. The symptom expression on differential hosts, the host range, and the properties of the viruses concerned were found to be sufficiently definite to permit identification and specific naming. Recently, certain other legume mosaic viruses have been studied for the purpose of accumulating evidence that beans, peas, and other leguminous plants may also be subject to a number of different viruses and that these viruses may be readily differentiated and identified in various ways. Additional data on the common bean mosaic virus (bean virus 1) and the yellow bean mosaic virus (bean virus 2) previously studied and the results of a comparative study of five other viruses affecting leguminous plants are presented in this paper.

REVIEW OF LITERATURE

A mosaic disease of sweet pea, *Lathyrus odoratus* L. described by Taubenhaus (28) in 1914 was shown by him to be transmissible by aphids and by artificial methods. Later reports on leguminous plant viruses by McLarty (13), Elliott (5), Dickson (3), Doolittle and Jones (4), Böning (1), Merkel (14), Zaumeyer (32), Zaumeyer and Wade (33), Henderson (9), Weimer (29), and Johnson (10) also were concerned primarily with accounts of symptoms and of transmission experiments. The actual identity of the viruses concerned was in most cases not established beyond the fact that they were found causing a mosaic on some particular host or hosts and were transmissible to certain others.

There are, on the other hand, reports of legume plant viruses in which the identity of the viruses was sufficiently established to per-

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² The author gratefully acknowledges his indebtedness to Dr. C. W. Hungerford for helpful advice given during the course of this investigation; to Dr. Merl Stubbs for helpful cooperation in the identification of certain viruses; to Drs. James Johnson and J. C. Walker for suggestions regarding the manuscript; to Dr. W. C. Snyder for supplying certain diseased specimens; and to Ruth Remaberg for photographs.

³ Reference is made by number (italic) to Literature Cited, p. 1038.

mit their recognition by other workers. Thus in the studies on common bean mosaic by Reddick and Stewart (22, 23, 24, 27), Fajardo (6, 7), Nelson (15), Pierce and Hungerford (18), and Pierce (17) it seems apparent that the same virus (bean virus 1) was used in all cases. Kendrick and Gardner (8, 11) established that a seed-borne soybean mosaic virus was nontransmissible to garden beans, 7 other species of *Phaseolus*, 2 species of *Dolichos*, field peas, and cowpeas. It would appear that this virus is quite specific to soybean, and not confusable with the yellow bean mosaic virus (bean virus 2) and an alfalfa mosaic virus (alfalfa virus 2) which Pierce (17) found transmissible to soybean as well as to bean and a number of other leguminous hosts. The two latter viruses (bean virus 2 and alfalfa virus 2) were studied (17) comparatively with the virus of common bean mosaic (bean virus 1) and all were found to be distinct and separate entities.

Osborn (16) has described a pea mosaic which he obtained from plants of *Vicia faba* L. The virus concerned produced distinctive enations on the under surfaces of infected crimson clover and pea leaves. Stubbs⁴ in an intensive study of viroses of the garden pea differentiated two distinct viruses capable of causing pea mosaic. One of these was found to produce enations similar to those described by Osborn (16). This virus was also found to infect the Perfection variety of peas, and was designated by Stubbs⁵ as enation pea mosaic (pea virus 1). The other virus was incapable of causing infection on Perfection peas, and was named pea virus 2.

Certain well-defined viruses commonly associated with nonleguminous plants have in some instances been found to affect plants of the family Leguminosae. Thus the tobacco ring spot virus was shown by Wingard (31) to be transmissible to bean and sweetclover. Pierce (17) extended the host range to include still other legume plants. Price (19) found that the ordinary tobacco mosaic virus (tobacco virus 1) would produce local lesions on bean, and Carsner (2) showed that the virus of curly top of sugar beets was transmissible to bean by means of the beet leaf hopper *Eutettix tenellus* (Baker). Severin and Henderson (25) reported common beans, lima beans, cowpeas, horsebeans, vetch, hairy Peruvian alfalfa, chickpea, and a number of clovers as susceptible to the curly top virus. In studying the host range of the virus of southern celery mosaic (celery virus 1), Wellman (30) found that following inoculation, broadbean (*Vicia faba*) developed small purplish primary lesions which did not become systemic. Price (20) found that certain strains of cucumber mosaic virus produced necrotic primary lesions on cowpea, *Vigna sinensis* (L.) Endl. One strain was found to cause a typical mosaic disease of cowpea.

Linford (12) reported the transfer of pineapple yellow spot from *Emilia sagittata* (Vahl) DC. to peas by *Thrips tabaci* Lindeman. Symptoms on peas were described as streak and as similar to symptoms noted on peas naturally infected with a streak.

It is evident from these accounts that there are a number of viruses capable of infecting leguminous plants, and that the mere observation of a virus infection on any particular legume host cannot necessarily

⁴ STUBBS, M. W. VIROSES OF THE GARDEN PEA (*PISEM SATIVUM* L.). Ph. D. thesis, University of Wisconsin. 1935. (Unpublished manuscript.)

⁵ STUBBS, M. W. See footnote 4.

be considered as a new and separate disease specific to that particular species.

EXPERIMENTAL MATERIALS, METHODS, AND HOSTS

VIRUSES STUDIED

The viruses studied in this investigation were obtained from various legume plants affected with viroses. The fact that many of the legume plants used were found to be susceptible to more than one virus makes it inaccurate and confusing to speak of the virus of the mosaic disease of red clover, for instance, without regard to which specific virus may be responsible for the virosis in question. An attempt has been made, therefore, to differentiate these viruses sufficiently to name them. In order to simplify the designation of the various viruses for the reader, the names have been applied at the beginning of the paper, rather than at the close. The viruses studied were as follows:

Common bean mosaic virus (bean virus 1).—This virus is the one responsible for the common and prevalent type of mosaic affecting common beans (*Phaseolus vulgaris*) and which has been described by Reddick and Stewart (22, 23, 24, 27), Fajardo (6, 7), Nelson (15), Pierce (17), and others. The virus was secured from mosaic-infected bean seedlings and was used in this study in inoculation tests on beans only.

Yellow bean mosaic virus (bean virus 2).—This virus was previously (17) described and named. In this study the virus was obtained from a mosaic-infected yellow sweetclover, (*Melilotus officinalis* (L.) Lam.) plant grown in the field at Moscow, Idaho, in 1934. Symptoms on bean and thermal death point studies established this as identical with the yellow bean mosaic virus (bean virus 2) previously studied.

White clover mosaic virus (white clover virus 1).—This virus was secured from a white clover (*Trifolium repens* L.) plant naturally infected with mosaic at Moscow, Idaho. The same virus was obtained also from naturally infected yellow trefoil. The virus is transmissible to beans, sweetclover, red clover, and peas. It causes necrosis of many varieties of peas. Differential hosts, thermal death point, and aging experiments are described in detail later in the paper.

Enation pea mosaic virus (pea virus 1).—This virus was obtained from peas, *Pisum sativum* L., naturally infected with mosaic in the field. The infected specimens were grown in California. This virus produces enations on the under surfaces of leaves of affected peas similar to those described by Osborn (16) and Stubbs.⁶ It is referred to in this paper as pea virus 1 since it is believed to be the same virus as that described by Stubbs.

Common pea mosaic virus (pea virus 3).—This virus was obtained from a mosaic pea seedling of the Horsford variety grown in the greenhouse of the Department of Plant Pathology, University of Idaho, Moscow, Idaho. The specificity of the virus was suspected from the fact that it was not transmissible to beans. As shown later, this virus is similar in symptom expression to a group of viruses studied by Stubbs⁶ as pea viruses 2 A, B, and C; but since certain differences

⁶ STUBBS, M. W. See footnote 4.

have been noted, the virus used in this study is designated as the common pea mosaic virus (pea virus 3).

Soybean mosaic virus (soybean virus 1).—This virus was secured from plants grown from mosaic-infected seed of the Midwest variety of soybeans (*Soja max* (L.) Piper) obtained from the Purdue Agricultural Experiment Station, LaFayette, Ind. As shown later, this virus is undoubtedly the same as the one described by Gardner and Kendrick (8, 11).

A virus obtained from red clover (*Trifolium pratense* L.) and which produces local necrotic lesions on the small-seeded broadbean (*Vicia faba* var. *minor*) has been studied in a preliminary way. For purposes of designation this virus is referred to in this paper as the broadbean local-lesion virus. Final naming is reserved until such time as the host range has been studied and a more complete description worked out.

METHODS AND TEST HOSTS

The studies were made in the greenhouse where the temperature was usually held at about 25° C. Inoculum was prepared by crushing leaves and stems of infected plants in a sterile mortar and then removing gross solids by straining through cheesecloth. Inoculations were made on young plants by rubbing the leaf surfaces with a cheesecloth pad that had been immersed in the inoculum. The percentage infection was greatly increased by the use of carborundum powder, as described by Rawlins and Tompkins (21). In these tests, however, the carborundum powder was added directly to the inoculum just prior to making inoculations.

The thermal death point determinations were made by pipetting 2 cc of a 1 to 1 dilution of freshly extracted infective juice into thin-walled test tubes. The tubes were stoppered and placed in an agitated constant-temperature water bath for 10 minutes at the desired temperature, after which the tubes were rapidly cooled in cold running water. Inoculations were then made immediately to various test hosts. The aging tests were made by storing inocula in stoppered test tubes in a darkened cupboard at a temperature of 20° to 22° C. The virus extracts were tested at desired intervals by removing about 2 cc from the storage tubes and inoculating young test plants in the usual manner.

The test hosts used were: A French variety of dwarf edible-pod peas secured from a commercial seed company at Moscow, Idaho, under the name "Nain mangetout à longue cosse" (*Pisum sativum* var. *saccharatum* Hort.); a mosaic-free strain of Stringless Refugee Green beans (*Phaseolus vulgaris*); a strain of small-seeded broadbeans (*Vicia faba* var. *minor*); yellow sweetclover (*Melilotus officinalis*); and the Midwest variety of soybeans (*Soja max*). Each of these hosts was found to be susceptible to one or more of the viruses studied. The thermal death point and aging determinations were usually made on two or more different hosts.

EXPERIMENTAL RESULTS

The differentiation of the viruses considered in this paper was based upon symptom expression, varietal susceptibility of peas and beans, susceptibility of certain leguminous plant species, and upon certain properties of the viruses *in vitro*.

SYMPTOM EXPRESSION

The differentiation of viruses on the basis of symptoms may often lead to erroneous conclusions, but as long as the ultimate test of virus infection must be based upon symptoms of one kind or another it is essential that careful attention be given to a comparative study of symptom expression on differential hosts. In this study symptom expression has not been confined to the principal host species but is also described on other hosts that have been found to be particularly well adapted to differentiation.

Common bean mosaic virus (bean virus 1) produces the common mottled and leaf curl symptom associated with the seed-borne type of bean mosaic as shown in figure 1, A, and as described in detail by Fajardo (6), Nelson (15), and Pierce (17).

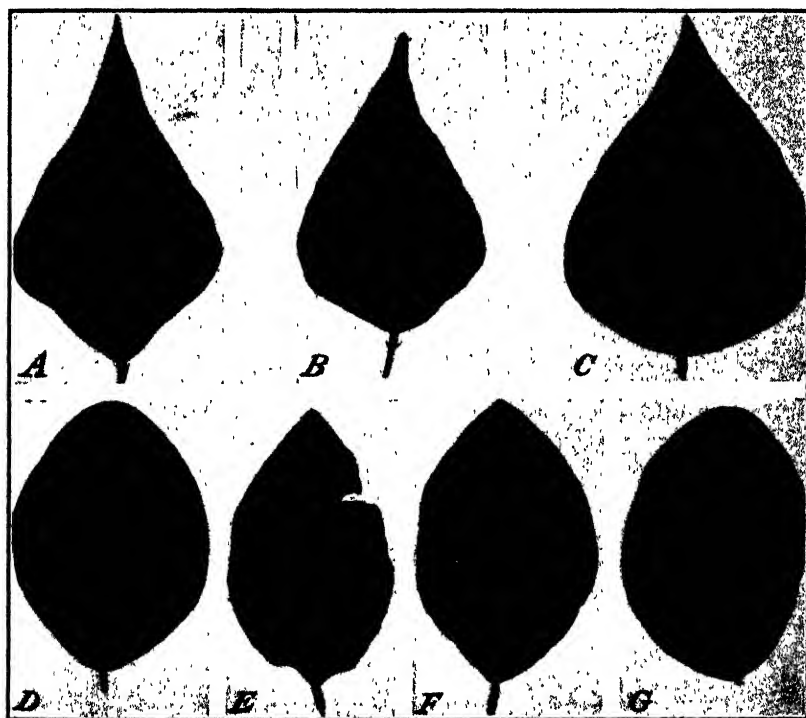


FIGURE 1.—Bean and soybean leaves showing symptoms of infection with certain viruses. A, Stringless Refugee Green bean infected with bean virus 1; B, Robust bean infected with bean virus 2; C, Robust bean infected with white clover virus 1; D, Midwest soybean, noninoculated control; E, Midwest soybean infected with soybean virus 1; F, Midwest soybean infected with pea virus 1; G, Midwest soybean infected with the broadbean local-lesion virus.

The symptoms caused by yellow bean mosaic virus (bean virus 2) were described fully on differential varieties of bean in a former publication (17); however, a few of the important symptoms of diagnostic value are repeated here. The first symptom following inoculation of the primary leaves on Stringless Refugee Green beans is a distinctive drooping of the developing trifoliate leaf. The leaflets are definitely pointed downward from the point of attachment to the petiole. Small light-yellow spots soon develop in the dark-green

background; and the yellowing gradually spreads over more or less of the surfaces of the young trifoliate leaves, producing a definite yellow mosaic pattern. Figure 1, *B*, shows typical symptoms on the Robust variety of beans. None of the other viruses tested on beans produced the distinctive downward pointing of the young trifoliate leaf.

On certain varieties of peas, bean virus 2 produces a typical mosaic pattern (fig. 2, *B*). The pea mosaic caused by this virus, however, was somewhat milder than the pea mosaics caused by pea virus 1 and pea virus 3, as shown in figure 2, *B*, *C*, and *D*.

The symptoms produced by enation pea mosaic virus (pea virus 1) on peas was usually very severe, and consisted of mottling, crinkling, and savoying of the leaves and stipules (fig. 3, *A*). On very susceptible varieties like Alderman, necrotic spots appear, accompanied by proliferations on the under surfaces of the leaves, as shown in figure 3, *B*. Since these enations were not found on any plants affected with other viruses they are believed to be of especial diagnostic value. The pea mosaic of Osborn (16) is undoubtedly the same as enation pea mosaic, since he found enations as a constant symptom. This virus is believed to be the same as that causing extreme malformation of pods as described by Snyder (26). This virus also affects soybeans, producing a mottled dark and light-green pattern as shown in figure 1, *F*.

Common pea mosaic virus (pea virus 3) causes a distinct mottling consisting of yellow and green patterns on peas. In figure 2, *C*, is shown typical mottling symptoms on Alaska peas. This virus also produces mosaic mottling on broadbean, red clover (fig. 4, *D*), and yellow sweetclover. No symptoms were produced on beans.

White clover mosaic virus (white clover virus 1) produces a very distinct mosaic on white clover, *Trifolium repens*. There is also some dwarfing and crinkling as shown in figure 5, *A*. The symptoms on red clover and yellow sweetclover are particularly severe. Distinct mottling is accompanied by crinkling of the leaves, and in some cases a slight spot necrosis is evident on red clover leaves (fig. 4, *B*). Yellow sweetclover plants are definitely dwarfed, crinkled, and mottled when infected with white clover virus 1.

Under winter greenhouse conditions a complete necrosis of peas was caused by this virus (fig. 5, *F*). This complete killing was due in part to secondary attack by damping-off fungi. Necrosis is caused on broadbeans, but usually the plants survive and show a distinct mottling together with some malformation and necrotic spots in the leaves (fig. 5, *E*). White clover virus 1 is also transmissible to beans and produces some local vein necrosis on Stringless Refugee Green, followed by a diffused yellow mottling in the subsequent developing leaves. This virus does not produce the distinctive drooping effect on the young trifoliate leaves which is characteristic of infections with bean virus 2. Figure 1, *C*, shows diffuse mottling on the Robust variety. Symptoms on alfalfa are mottling and malformation as shown in figure 5, *C*.

Soybean mosaic caused by soybean virus 1 is characterized by mottling of a more or less mild type under greenhouse conditions. The leaves are usually curled downward and malformed with dark-green areas interspersed over a light-green (chlorotic) background. Figure 1, *E*, shows typical leaf symptoms following inoculation of the

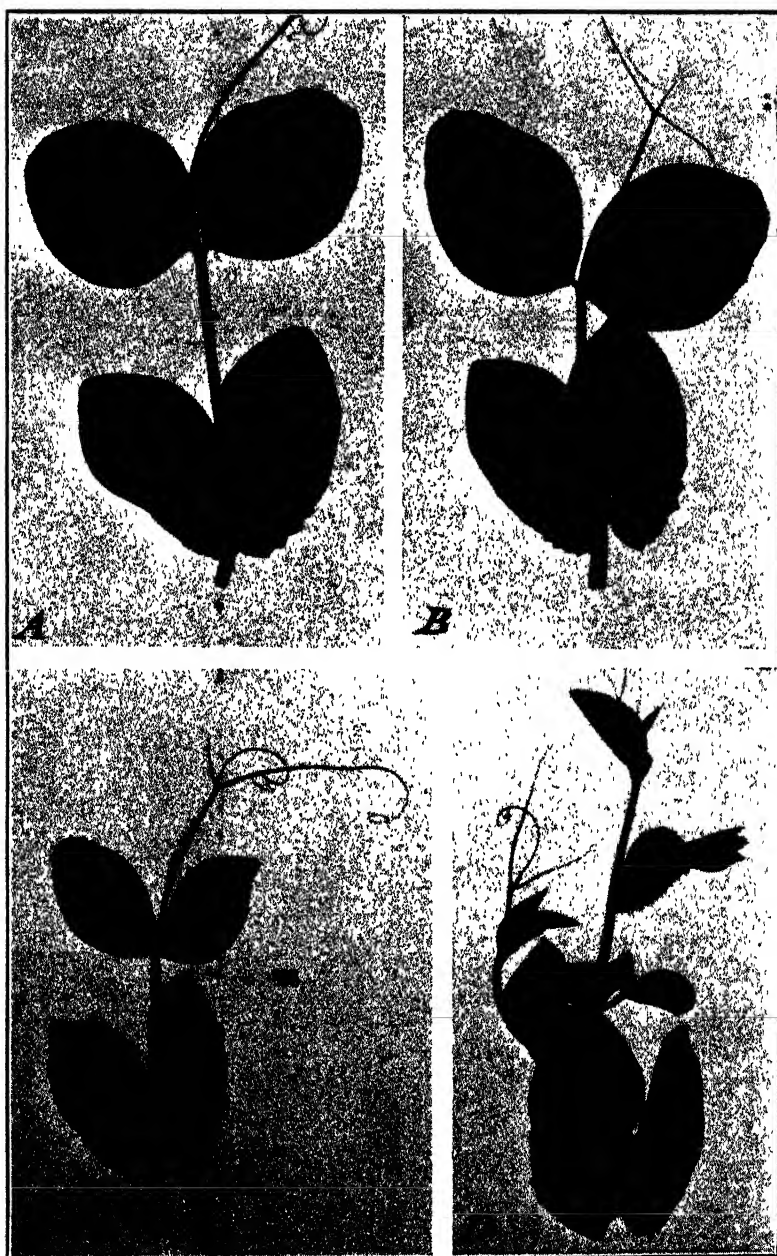


FIGURE 2.—Symptoms produced by three different viruses on Alaska peas: *A*, Noninoculated control; *B*, infected with bean virus 2; *C*, infected with pea virus 3; *D*, infected with pea virus 1. Note twisting and malformation characteristic of infections with virus used in *D*.



FIGURE 3.—Symptoms on peas produced by infection with pea virus 1: *A*, Naturally infected mosaic pea plant from which pea virus 1 was obtained; *B*, lower surfaces of leaflets showing enations produced by pea virus 1.

Midwest variety of soybean. The symptoms and effect on yield have been fully described by Gardner and Kendrick (8, 11).

The broadbean local-lesion virus produces distinct local necrotic lesions on the small-seeded broadbean, *Vicia faba* var. *minor* (fig. 6, A, B). These lesions are a dark chocolate brown in color. The virus did not become systemic on broadbean. On peas it caused a necrosis of the veinlets which was followed by a distinct chlorosis and dropping of the leaves. In figure 6, D and E, are shown typical symptoms on Progress and Horal varieties of peas. The symptoms appear first on the lower leaves and then progressively upward, in

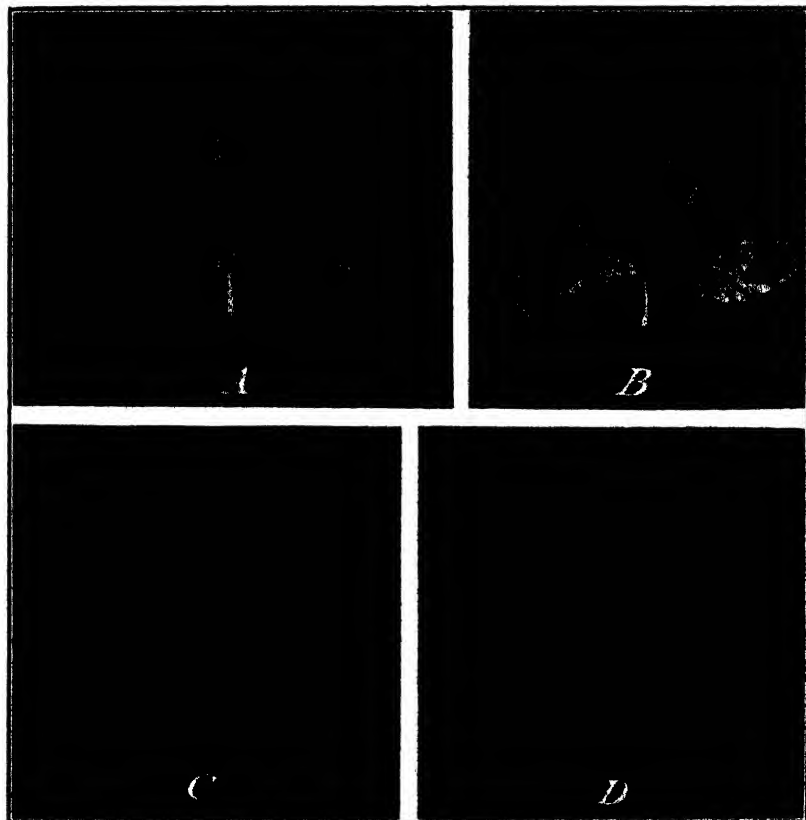


FIGURE 4.—Leaf symptoms produced by three different viruses on red clover: A, Noninoculated control; B, infected with white clover virus 1; extreme crinkling and malformation is characteristic; C, infected with bean virus 2; D, infected with pea virus 3. Note that symptoms produced by bean virus 2 and pea virus 3 are very similar.

some cases killing the plants. More often, however, the disease does not affect the upper part of the plants. On soybean this virus produces a slight chlorosis, as shown in figure 1, G.

RESISTANCE AND SUSCEPTIBILITY IN PEA VARIETIES

Each of the viruses studied was tested on the following varieties of garden peas, *Pisum sativum*: Alaska, Perfection, Wisconsin Early Sweet, Green Admiral, Surprise, Progress, Alderman, Dwarf Tele-

phone, and Horal. These tests were made more for the purpose of establishing differences in the viruses than in showing differences in varietal susceptibility. However, it will be seen that certain very

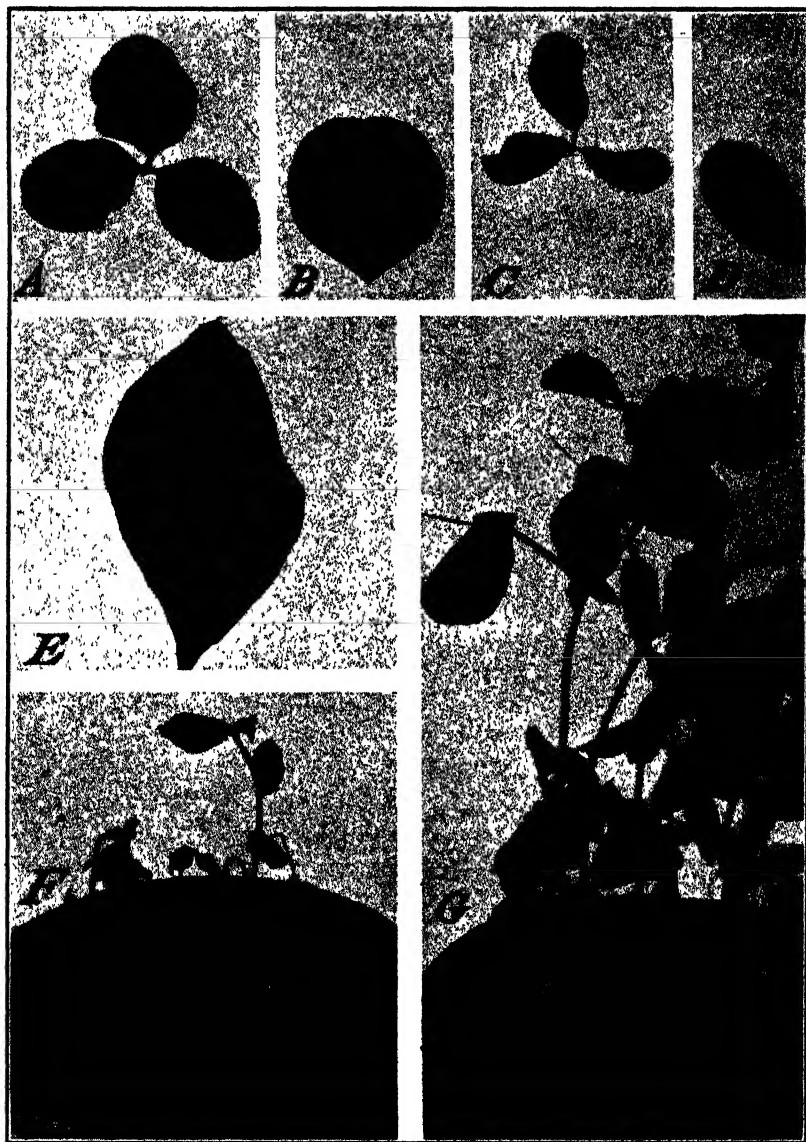


FIGURE 5.—Symptoms on various hosts produced by white clover virus 1: *A*, Infected white clover; *B*, noninoculated white clover; *C*, infected common alfalfa; *D*, noninoculated alfalfa; *E*, infected small-seeded broadbean; *F*, infected Nain mangetout à longue cosse variety of peas showing complete necrosis; *G*, noninoculated peas.

definite differences in varietal susceptibility were established, which may be of value to plant breeders seeking to develop resistant varieties.

Inoculations were made in the regular routine manner; carborundum powder was added directly to the inoculum and the leaf surfaces were then rubbed with a cheesecloth pad which had been immersed in the inoculum. No attempts were made to reinoculate plants; thus in certain instances in which varieties were not 100 percent infected, it cannot necessarily be concluded that the remainder were entirely resistant, but rather that they merely escaped infection. In table 1 the number of plants inoculated of each variety is given together with the percentage infection obtained with the various viruses.

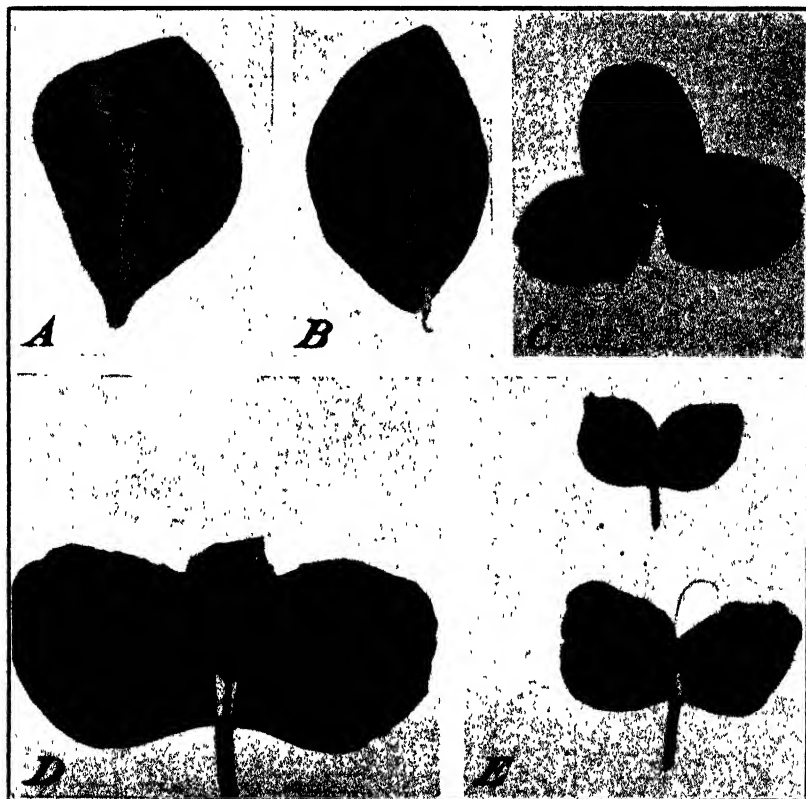


FIGURE 6.—Symptoms on various hosts produced by the broadbean local-lesion virus: A, broadbean control, inoculated with water; B, local lesions on broadbean; C, red clover infected with both the broadbean local-lesion virus and bean virus 2; D, Progress pea leaves infected with the broad bean local lesion virus; E, Horal pea leaves infected with broadbean local-lesion virus, note necrosis of veinlets.

In a previous paper (17) it was reported that the variety Perfection was resistant to infection with bean virus 2. The same was found to be true in the present investigation; however, certain other varieties of peas have been found to be susceptible. Alaska, Green Admiral, Alderman, and Dwarf Telephone were all found to be very susceptible (table 1). No infection was secured on Perfection and Horal varieties.

Pea virus 1 was transmitted to all of the varieties of peas tested (table 1). The varieties Perfection and Horal, which were found

resistant to pea virus 3, were susceptible to infection with pea virus 1. On varieties susceptible to both viruses, however, lower percentages of infection were secured with pea virus 1 than with pea virus 3.

The pea varieties tested for susceptibility to pea virus 3 were in general more susceptible to it than to bean virus 2; however, the most susceptible varieties to bean virus 2 were also the most susceptible to pea virus 3. Alaska, Alderman, Dwarf Telephone, and Green Admiral were readily infected (table 1). No infection was obtained on Horal and only three plants of Perfection were infected. These three plants may well have been admixtures or rogues.

White clover virus 1 produced necrosis of all the pea varieties tested. On the basis of percentage of plants infected, Horal and Perfection exhibited the greatest resistance. One hundred percent infection was obtained on many varieties (table 1). Infection with this virus appeared to predispose plants to attack by damping-off fungi.

No infection was obtained with soybean virus 1 upon the varieties of peas (*Pisum sativum*) tested. Kendrick and Gardner (11) were unable to obtain infection on field peas in their trials.

The broadbean local-lesion virus was successfully transmitted to all the varieties of peas tested (table 1). Only a low percentage of infection was obtained on Perfection and Wisconsin Early Sweet. In these limited tests Progress was the most readily infected.

RESISTANCE AND SUSCEPTIBILITY IN BEAN VARIETIES

Six varieties of beans, *Phaseolus vulgaris*, were tested for susceptibility to the various viruses. The varieties tested were: Stringless Refugee Green, Idaho Refugee, Wisconsin Refugee, Robust, Common Great Northern, and Great Northern UI No. 1. The data are given in table 1.

Stringless Refugee Green and Common Great Northern were susceptible to the common bean mosaic virus (bean virus 1). No infection with this virus was obtained on Robust, Great Northern UI No. 1, Idaho Refugee, and Wisconsin Refugee.

All varieties were more or less susceptible to the yellow bean mosaic virus (bean virus 2). The varieties Robust, Great Northern UI No. 1, Idaho Refugee, and Wisconsin Refugee which were resistant to common bean mosaic were found to be less easily infected with bean virus 2 than was Stringless Refugee Green. These results are in accord with previous findings (17).

All of the bean varieties tested were susceptible to infection with white clover mosaic virus (white clover virus 1). Higher percentages of infection were obtained on Stringless Refugee Green than on any of the other varieties. The symptoms produced by this virus were considerably less severe than those produced by bean virus 2.

Enation pea mosaic virus (pea virus 1) was not transmissible to any of the bean varieties tested.

No infection on bean varieties was obtained with the common pea mosaic virus (pea virus 3). Inoculum from inoculated beans when transferred back to peas gave no infection, showing that the virus was not present and that the beans were immune from infection with this virus.

Inoculations to the six varieties of beans with the common soybean mosaic virus (soybean virus 1) failed to produce symptoms. Transfer

inoculations from inoculated beans back to soybean gave no infection, indicating that the virus was not present in beans.

Only the Stringless Refugee Green variety of beans was tested for susceptibility to the broadbean local-lesion virus. In no case was infection obtained on this variety.

RESISTANCE AND SUSCEPTIBILITY OF CERTAIN PLANT SPECIES

The following plant species were tested by artificial inoculation for susceptibility and resistance to each of five viruses: Small-seeded broadbean, *Vicia faba* var. *minor*; yellow sweetclover *Melilotus officinalis*; red clover, *Trifolium pratense*; white clover, *T. repens*; common alfalfa *Medicago sativa* L.; Midwest variety of soybean, *Soja max*; Stringless Refugee Green bean, *Phaseolus vulgaris*; Nain mangetout à longue cosse variety of peas, *Pisum sativum* var. *saccharatum*; Connecticut Havana No. 38 tobacco, *Nicotiana tabacum* L.; and garden petunia, *Petunia hybrida* Vilm.

TABLE 2.—Summarized scheme for the differentiation of certain viruses affecting legume plants, based on data secured on greenhouse determinations of the resistance and susceptibility of certain plant species¹

Virus	<i>Phaseolus vulgaris</i>		<i>Pisum sativum</i>		<i>Melilotus officinalis</i> , yellow sweet-clover	<i>Soja max</i> , Midwest soybean
	Stringless Refugee Green	Robust	Alaska	Perfection		
Bean virus 1.....	+++	—	—	—	—	—
Bean virus 2.....	+++	++	++	—	++	+
Pea virus 1.....	—	—	++	++	++	++
Pea virus 3.....	—	—	+++	—	++	—
White clover virus 1.....	+++	+	+++	+++	+++	—
Soybean virus 1.....	—	—	—	—	—	+++
Broadbean local-lesion virus.....	—	—	++	++	(²)	+++

¹ +++ designates severe infection, ++ moderate infection, + slight infection, and — designates no infection.

² No data.

It was the purpose of these tests to establish, if possible, differences in the host range of each virus. Obviously the limited number of species tested cannot be considered as adequate host-range studies; however, it will be seen that the differential susceptibilities of the various species to the different viruses is of value in establishing virus identities (tables 1 and 2). Thus the pea mosaic viruses, pea virus 1 and pea virus 3, are seen to differ from most of the other viruses in being nontransmissible to bean. Pea virus 3 differs from pea virus 1 in being nontransmissible to the Perfection and Horal varieties of peas. Furthermore, in these tests pea virus 3 was not successfully transmitted to soybean, while pea virus 1 was transmitted readily. The broadbean local-lesion virus produced on broadbean only local necrotic lesions, thus differentiating it from those viruses causing systemic infection on broadbean. The host range of the broadbean local-lesion virus has not been studied. Of the five viruses used in tests with tobacco and petunia, none was transmitted, thus establishing that they were different from the many viruses known to affect tobacco and petunia of the family Solanaceae. Many other important differences in the susceptibility of the various species may be observed

from the results given in table 1. As a further aid in the identification of legume plant viruses, a summarized scheme for their differentiation on the basis of host susceptibility and resistance is given in table 2. As shown in this table, many of the viruses may be readily identified by making artificial inoculations to a few varieties of peas and beans and to yellow sweetclover and soybean. In a previous paper (17) it was reported that red clover was not susceptible to bean virus 2, but in the present investigation in which carborundum powder was used as an abrasive, a low percentage of infection was obtained on red clover. Furthermore, a number of red clover plants brought in from the field were found to be naturally infected with bean virus 2. It is to be expected that as more adequate methods of artificial inoculation are developed certain other species listed as resistant to certain viruses will be found to be susceptible. It is probable also that the natural insect vectors may extend the list of susceptible host plants.

PROPERTIES OF THE VIRUSES

The thermal inactivation points and resistance to aging in vitro were determined for all of the viruses studied in the hope of finding differences sufficient for a basis of separation and identification. The determinations on each virus were made in most cases on two or more different hosts. Thus the virus of common pea mosaic (pea virus 3) was tested on peas and on broadbean. Wherever possible the inoculum was secured from the same host species as that upon which the determinations were made. The results are tabulated in tables 3, 4, and 5.

THERMAL INACTIVATION POINT

(Tables 3 and 4)

The thermal inactivation point of bean virus 2 was found to lie between 58° and 60° C. when heated for 10 minutes. It was previously reported (17) at 56° to 58°, but in the present investigation an occasional infection was obtained with virus extracts heated at 58°.

Pea virus 1 was usually inactivated at 56° C., but in two trials infection was obtained after heating at this temperature. No infections were obtained in any trial after heating at 58° for 10 minutes.

Pea virus 3 was inactivated at 62° to 64° C. for 10 minutes in the determinations made on both peas and broadbeans.

White clover virus 1 was found not to survive the 58° C. heatings in tests on sweetclover and peas. In one test on beans this virus gave a low percentage of infection following heating at 58°.

The thermal death point determinations on the broadbean local-lesion virus were based on the ability of the virus to produce local lesions on broadbean. This virus produced few lesions following heating at 60° C. and no lesions after heating at 62° (table 4).

Soybean virus 1 was inactivated by heating at 58° C. in all tests on the Midwest variety of soybean.

TABLE 3.—Comparison of the thermal inactivation points of white clover virus 1, pea virus 1, pea virus 3, bean virus 2, and soybean virus 1 as determined by systemic infection on various hosts

Temperature (° C.)	White clover virus 1 on—								Pea virus 1 on—		Pea virus 3 on—				Bean virus 2 on—		Soybean virus 1 on—	
	Peas		Broad-bean		Yellow sweet-clover		Beans		Peas	Peas	Broad-bean		Beans	Soy-bean				
	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected	Plants in-oculated	Plants infected		
Inoculated control....	No. 8	No. 8	No. 8	No. 8	No. 10	No. 10	No. 16	No. 16	No. 30	No. 23	No. 16	No. 14	No. 15	No. 12	No. 21	No. 19	No. 45	No. 38
50.....	---	---	---	---	5	5	5	5	---	---	---	---	---	---	---	---	---	---
54.....	---	---	8	7	---	---	---	---	25	8	---	---	---	---	---	---	---	---
56.....	8	8	8	3	5	5	16	8	25	2	---	---	---	---	18	13	22	15
58.....	8	0	8	0	10	0	16	1	30	0	10	1	15	6	20	2	22	16
60.....	8	0	8	0	10	0	16	0	32	0	16	1	15	4	16	0	30	0
62.....	---	---	---	---	---	---	---	---	25	0	10	1	15	1	---	---	---	---
64.....	---	---	---	---	---	---	---	---	---	---	10	0	10	0	---	---	---	---

TABLE 4.—The thermal inactivation point and resistance to aging in vitro of the broadbean local-lesion virus as determined by the production of local lesions on *Vicia faba* var. *minor*¹

Temperature (° C.)	Test 1	Test 2	Test 3	Test 4	Time aged	Test 1	Test 2
	Number	Number	Number	Number	Hours	Number	Number
Inoculated control.....	480	660	213	208	0	480	213
50.....		610			24	146	120
56.....					30	30	
58.....	6		10		48	20	5
60.....	0	6	1	9	72	0	0
62.....	0		0	0			
65.....		0					

¹ Numbers are total lesions produced on 20 inoculated leaves of *Vicia faba* var. *minor*.

TABLE 5.—Comparative tests on resistance to aging in vitro of white clover virus 1, pea virus 1, pea virus 3, bean virus 2, and soybean virus 1, as determined by systemic infection on various hosts

[illegible]

The difference in the thermal inactivation points of the various viruses, while in most cases small, were in some instances sufficient to be of value in comparative differentiation.

RESISTANCE TO AGING IN VITRO

(Tables 4 and 5)

Bean virus 2 was inactivated after aging 24 to 48 hours, which was in accord with previous findings (17).

Pea virus 1 was found to be noninfectious after aging 3 days.

Pea virus 3 was still infectious after aging 48 hours but failed to infect either peas or broadbeans after 72 hours.

White clover virus 1 withstood agings of 5 days, but after this length of time symptoms were slower in developing than in the control tests, indicating that probably the concentration of infective virus had been considerably reduced. No infection was obtained after aging 7 days.

The broadbean local-lesion virus and soybean virus 1 were both inactivated by aging 3 days.

The differences in the ability of certain of the viruses to withstand aging in vitro are sufficient in most cases to show definitely that separate and distinct viruses were concerned.

EXPERIMENTS ON VIRUS SEPARATION AND COMBINATION

A red clover plant taken from the field in 1934 was found to be infected with two separate and distinct viruses. One of these was the broadbean local-lesion virus (fig. 6, *B*) and the other has been identified as bean virus 2 (fig. 4, *C*). These conclusions were arrived at in the following manner: The original virus infection on red clover produced mottling accompanied by some necrosis as shown in figure 6, *C*. When the infected red clover was used as a source of inoculum and inoculations were made directly to the Nain mangetout à longue cosse variety of peas, a necrosis and streaking developed as shown in figure 7, *A*. When inoculations were made direct from red clover to the small-seeded broadbean, local necrotic lesions developed within 4 or 5 days; and 4 to 6 days later systemic symptoms appeared in the new growth. Inoculations to peas with the systemic mosaic material from broadbean as inoculum caused only a mild mottling in peas, as shown in figure 7, *B*. It seemed probable, therefore, that on broadbeans the local lesions were caused by one virus and the systemic infection by another, and that the combination of the two viruses on peas produced necrosis. The systemic entity on broadbean was studied alone, and in tests on Stringless Refugee Green beans appeared to be identical with bean virus 2. The original combination from red clover produced only systemic infection typical of that produced by bean virus 2 in comparative inoculations to bean. Furthermore, inoculations from bean back to broadbean produced only systemic infections, indicating that beans were not susceptible to the local-lesion entity.

The isolation of the local-lesion entity from the combination on red clover was accomplished by making inoculations to the Horal or Perfection varieties of peas which are resistant to bean virus 2 but susceptible to the local-lesion virus. Figure 6, *E*, shows Horal peas infected with the local-lesion virus alone.

DISCUSSION

The purpose of the work described in this paper has been primarily to differentiate certain viruses that affect leguminous plants. It is hoped ultimately to work out a more complete description of each virus which will include host range, properties, transmissibility through seed, insect vectors, and occurrence in nature on economic hosts. In the meantime it appeared essential to develop short methods of identification, so that the virus causing a particular mosaic disease, could be readily identified by making artificial inoculations to a few differential hosts or by property tests. The necessity for such a method of identification can readily be appreciated from the frequent use in the literature of such terms, for instance, as the virus of red clover mosaic, without recognition of the fact that there is or might be anyone of several viruses capable of causing a mosaic



FIGURE 7.—Symptoms produced on Nain mangetout à longue cosse peas: A, By combination of bean virus 2 and the broadbean local-lesion virus; B, by bean virus 2 alone.

symptom on red clover. It is realized that the identification of certain viruses will, in many instances, be a difficult task, especially in cases where two or more viruses are present in the same plant. On the whole, however, it is believed that more careful attention on the part of workers dealing with legume mosaics to the identification of the particular viruses concerned will go far toward clearing up the confusion existing in this particular field of virus study.

A few of the important facts pertaining to various viruses known to affect peas and beans have been brought together in table 6, not only to show that these hosts may be subject to several different viruses, but also to aid in the identification of the viruses. Data on certain viruses reported by other workers are included. Under the heading, "Differential hosts and diagnostic characteristics", at least one

TABLE 6.—A partial list of viruses capable of infecting beans and peas, with notations on certain characteristics and properties useful in their differentiation and identification

Host and virus	Host symptom	Thermal death point	Longevity in vitro	Differential hosts and diagnostic characteristics	Authority
Common bean, <i>Phaseolus vulgaris</i> : Bean virus 1.....	Leaf curl and mottling.....	° C. 56-58	Days 1	Seed-transmitted in bean. Robust. Great Northern U I No. 1, Idaho Refugee, and Wisconsin Refugee, immune. Pierce (17), this paper.	Fajardo (6, 7), Nelson (16), Pierce (17).
Bean virus 2.....	Yellow mosaic.....	58-60	1 to 2	Not seed-transmitted in bean. Foreigning bean varieties susceptible. Pierce (17), this paper.	Pierce (17), this paper.
White clover virus 1.....	Slight local necrosis and mild mosaic.....	58	6 to 7	Necrosis of peas. Severe mosaic on sweetclover, red clover, and white clover.	This paper.
Alfalfa virus 2.....	Local necrotic lesions.....	62-64	7 to 9	Local lesions on many varieties of bean, including Red Valentine.	(17), Zaunmeyer and Wade (37).
Tobacco virus 1.....	Small local lesions.....	90	(1)	Systemic on tobacco. Local lesions on <i>Nicotiana glauca</i> and certain varieties of bean. Red Valentine immune.	Pierce (19), Pierce (17).
Tobacco ring spot virus.....	Local and systemic necrosis.....	66	7 to 9	Ring spot symptom on tobacco.....	Wingard (31), Pierce (17).
Garden pea, <i>Pisum sativum</i> : Pea virus 1.....	Mottling, distortion, and enations.....	53	2 to 3	Not transmissible to bean. Perfection and Horal peas and Midwest soybean susceptible.	O s b o r n (16), Stubbs, this paper.
Pea virus 2A, B, C.....	A, marble; B, speckle; C, mild.....		3½ to 1	Not transmissible to bean, soybean, red clover, or Perfection peas.	Stubbs, this paper.
Pea virus 3.....	Yellow mosaic mottling.....	62-64	2 to 3	Not transmissible to beans, soybeans, or Horal peas. Transmissible to red clover. Rarely seed-transmitted.	This paper.
White clover virus 1.....	Necrosis.....	58	6 to 7	(See under bean)	Do.
Broadbean local-lesion virus.....	Veinlet necrosis, streak.....	60-62	2 to 3	Local lesions on small-seeded broadbean. Not transmissible to beans.	Do.
Alfalfa virus 2.....	Top necrosis on Perfection.....	62-64	7 to 9	Local lesions on bean. Necrosis on Perfection peas.	Pierce (17).
Tobacco ring spot virus.....	Top necrosis.....	66-70		Ring spot symptom on tobacco.....	Stubbs, this paper.
Phaseolus yellow spot virus.....	Streak.....			Transmitted by <i>Trips tabaci</i> .	Linford (16).
Bean virus 2.....	Mild mosaic.....	58-60	1 to 2	(See under bean)	This paper.

1 3 months or more.

2 Stubbs, M. W. See footnote 4.

characteristic is given wherein a specific virus differed from the other viruses affecting the same host. Certain differences in the thermal death points and resistance to aging in vitro also are of value in identifying some of the viruses. It should be pointed out, however, that the thermal death points of most of the legume mosaic viruses studied were grouped rather closely around 60° C. This fact may be of significance in the classification of viruses; that is, if the thermal death points of certain groups of viruses are found to be correlated to some extent with the type of host plant affected, a more or less natural grouping may eventually be made. With a view to eventual classification, it would appear advantageous to consider the legume mosaic viruses, the cane-fruit mosaic viruses, the solanaceous mosaic viruses, and so forth, each as separate groups; and to separate and differentiate the viruses and the virus strains within each group by extensive and intensive comparative studies as to plant species and horticultural varieties susceptible, transmissibility by insects and by artificial methods, transmissibility through seed, and physical properties.

The transmissibility of the various viruses through seed was not studied. However, a large number of seedlings of various varieties of peas were grown for inoculation purposes, and it seems significant that only a single case of seed transmission was observed throughout the course of the study. The virus obtained in this one case of seed transmission was studied and named common pea mosaic virus (pea virus 3).

It may be well to consider here the possible identity of certain viruses referred to in the literature. The pea mosaic described by Doolittle and Jones (4) and the broadbean mosaic of Böning (1) would seem on the basis of host relationship to have been caused by the same virus as the one described in this paper as pea virus 3. This may also have been the same virus that Zaumeyer and Wade (33) noted on red clover and that they found to be nontransmissible to bean. Stubbs' was unsuccessful in transmitting his pea virus 2 A, B, C to red clover, and therefore considered his strains as distinct from those of Doolittle and Jones (4), Böning (1), and Zaumeyer and Wade (33). It must be admitted, however, that the symptom expression of Stubbs' pea virus 2A is strikingly similar to that of pea virus 3. There can be little question but that the pea mosaic virus of Osborn (16) was the same as the virus later described by Stubbs and referred to in this paper as pea virus 1.

The viruses affecting certain legume hosts which Zaumeyer and Wade (33) found to be transmissible to beans may have been either bean virus 2, white clover virus 1, or both. The alfalfa mosaic virus of Zaumeyer and Wade (33) which produced only necrotic local lesions on bean was probably the same virus as that described by the writer in a previous paper (17) as alfalfa virus 2. The alfalfa mosaic virus which Weimer (29) found to be nontransmissible by artificial means should in all probability be considered as a separate and distinct virus. Henderson's (9) new virosis of sweetclover which he found to be transmissible to tobacco and petunia but which differed from the tobacco ring spot virus, must have been caused by a virus distinct from any studied in this investigation since none of the latter was found to be transmissible to tobacco.

⁷ STUBBS, M. W. See footnote 4.

The broadbean local-lesion virus described in this paper which tends to produce streak symptoms on peas, especially when in combination with other viruses, may or may not be the same as Linford's (12) streak. A further study of the host range of this virus would undoubtedly give significant evidence one way or the other.

It seems to be sufficiently evident from these investigations that many leguminous species are susceptible to several different viruses. Further progress in and understanding of the viroses of legumes is dependent, therefore, upon recognition of this fact, and upon comprehensive studies of the viruses themselves as well as of the diseases caused by them.

SUMMARY

The investigations reported in this paper were concerned primarily with the differentiation and identification of seven viruses affecting leguminous plants. The viruses studied were: The common bean mosaic virus (bean virus 1); the yellow bean mosaic virus (bean virus 2); a white clover mosaic virus (white clover virus 1); enation pea mosaic virus (pea virus 1); common pea mosaic virus (pea virus 3); the common soybean mosaic virus (soybean virus 1); and a virus obtained from red clover which was designated as the broadbean local-lesion virus. Differentiation was based upon symptom expression on differential hosts, varietal susceptibility of peas and beans, susceptibility of certain leguminous plant species, and upon certain properties of the viruses *in vitro*.

For practical purposes of identification the most significant differences between viruses was found in their host ranges and in varietal susceptibility of peas and beans. Pea viruses 1 and 3 were not transmissible to beans by the artificial inoculation methods used. Differentiation of pea virus 1 from pea virus 3 was made on the basis of resistance and susceptibility of pea varieties and upon differences in host range. Pea virus 1 infected soybean while pea virus 3 did not.

Bean virus 1 and bean virus 2 were readily differentiated on the basis of susceptibility and resistance of bean varieties. Bean virus 2 was transmissible to certain varieties of peas.

White clover virus 1 was differentiated from the other viruses studied on the basis of its ability to infect all varieties of beans and peas tested, and on the basis of symptom expression.

The broadbean local-lesion virus differed from all the other viruses studied in its ability to produce local necrotic lesions at the point of inoculation on the small-seeded broadbean.

Soybean virus 1 appeared to be specific to soybean, no infection on other species being obtained.

The differences in host ranges of the various viruses were supported in part by differences in symptom expression, differences in longevity *in vitro*, and in some cases by slight differences in the thermal death points of the viruses.

The differences established were believed to be sufficient to allow for the recognition of these viruses by other investigators.

LITERATURE CITED

- (1) BÖNING, K.
1927. DIE MOSAIKKRANKHEIT DER ACKERBOHNE (*VICIA FABA* L.) EIN BEITRAG ZU DEM MOSAIK DER PAPILIONACEEN. *Forschung Gebiete Pflanzenkrank. u. Immunität im Pflanzenreich* 4: [43]-111, illus.
- (2) CARLSNER, E.
1926. SUSCEPTIBILITY OF THE BEAN TO THE VIRUS OF SUGAR-BETT CURLY-TOP. *Jour. Agr. Research* 33: 345-348, illus.
- (3) DICKSON, B. T.
1922. STUDIES CONCERNING MOSAIC DISEASES. MacDonald Col. (Quebec) Tech. Bull. 2, 125 pp., illus.
- (4) DOOLITTLE, S. P., and JONES, F. R.
1925. THE MOSAIC DISEASE IN THE GARDEN PEA AND OTHER LEGUMES. *Phytopathology* 15: [763]-772, illus.
- (5) ELLIOTT, J. A.
1921. A MOSAIC OF SWEET AND RED CLOVERS. *Phytopathology* 11: 146-148, illus.
- (6) FAJARDO, T. G.
1930. STUDIES ON THE MOSAIC DISEASE OF THE BEAN (*PHASEOLUS VULGARIS* L.) *Phytopathology* 20: 469-494, illus.
- (7) ———
1930. STUDIES ON THE PROPERTIES OF THE BEAN-MOSAIC VIRUS. *Phytopathology* 20: 885-888.
- (8) GARDNER, W. M., and KENDRICK, J. B.
1921. SOYBEAN MOSAIC. *Jour. Agr. Research* 22: 111-114, illus.
- (9) HENDERSON, R. G.
1934. OCCURRENCE OF TOBACCO RING-SPOT-LIKE VIRUSES IN SWEET CLOVER. *Phytopathology* 24: 248-256, illus.
- (10) JOHNSON, E. M.
1923. A RINGSPOT-LIKE VIRUS DISEASE OF RED CLOVER. (Phytopath. Note) *Phytopathology* 23: 746-747, illus.
- (11) KENDRICK, J. B., and GARDNER, W. M.
1924. SOYBEAN MOSAIC: SEED TRANSMISSION AND EFFECT ON YIELD. *Jour. Agr. Research* 27: 91-98.
- (12) LINFORD, M. B.
1931. STREAK, A VIRUS DISEASE OF PEAS TRANSMITTED BY THRIPS TABACI. (Abstract) *Phytopathology* 21: 999.
- (13) McLARTY, H. R.
1920. A SUSPECTED MOSAIC DISEASE OF SWEET CLOVER. (Phytopath. Note) *Phytopathology* 10: 501-503, illus.
- (14) MERKEL, L.
1929. BEITRÄGE ZUR KENNTNIS DER MOSAIKKRANKHEIT DER FAMILIE DER PAPILIONACEEN. *Ztschr. Pflanzenkrank.* 39: [289]-347, illus.
- (15) NELSON, R.
1932. INVESTIGATIONS IN THE MOSAIC DISEASE OF BEAN (*PHASEOLUS VULGARIS* L.). *Mich. Agr. Expt. Sta. Tech. Bull.* 118, 71 pp., illus.
- (16) OSBORN, H. T.
1935. INCUBATION PERIOD OF PEA MOSAIC IN THE APHID, *MACROSIPHUM PISI*. *Phytopathology* 25: 160-177, illus.
- (17) PIERCE, W. H.
1934. VIROSES OF THE BEAN. *Phytopathology* 24: 87-115, illus.
- (18) ——— and HUNGERFORD, C. W.
1929. SYMPTOMATOLOGY, TRANSMISSION, INFECTION, AND CONTROL OF BEAN MOSAIC IN IDAHO. *Idaho Agr. Expt. Sta. Research Bull.* 7, 37 pp., illus.
- (19) PRICE, W. C.
1930. LOCAL LESIONS ON BEAN LEAVES INOCULATED WITH TOBACCO MOSAIC VIRUS. *Amer. Jour. Bot.* 17: 694-702, illus.
- (20) ———
1934. ISOLATION AND STUDY OF SOME YELLOW STRAINS OF CUCUMBER MOSAIC. *Phytopathology* 24: 743-761, illus.
- (21) RAWLINS, T. E., and TOMPKINS, C. M.
1934. THE USE OF CARBORUNDUM AS AN ABRASIVE IN PLANT-VIRUS INOCULATIONS. (Abstract) *Phytopathology* 24: 1147.

- (22) REDDICK, D., and STEWART, V. B.
1918. VARIETIES OF BEANS SUSCEPTIBLE TO MOSAIC. *Phytopathology* 8: 530-534.
- (23) ——— and STEWART, V. B.
1919. ADDITIONAL VARIETIES OF BEANS SUSCEPTIBLE TO MOSAIC. *Phytopathology* 9: 149-152.
- (24) ——— and STEWART, V. B.
1919. TRANSMISSION OF THE VIRUS OF BEAN MOSAIC IN SEED AND OBSERVATIONS ON THERMAL DEATH-POINT OF SEED AND VIRUS. *Phytopathology* 9: 445-450.
- (25) SEVERIN, H. H. P., and HENDERSON, C. F.
1928. SOME HOST PLANTS OF CURLY TOP. *Hilgardia* 3: 339-392, illus.
- (26) SNYDER, W. C.
1934. POD DEFORMATION OF MOSAIC-INFECTED PEAS. (Phytopath. Note) *Phytopathology* 24: 78-80, illus.
- (27) STEWART, V. B., and REDDICK, D.
1917. BEAN MOSAIC. (Abstract) *Phytopathology* 7: 61.
- (28) TAUBENHAUS, J. J.
1914. THE DISEASES OF THE SWEET PEA. *Del. Agr. Expt. Sta. Bull.* 106, 93 pp., illus.
- (29) WEIMER, J. L.
1934. STUDIES ON ALFALFA MOSAIC. *Phytopathology* 24: 239-247, illus.
- (30) WELLMAN, F. L.
1934. IDENTIFICATION OF CELERY VIRUS I, THE CAUSE OF SOUTHERN CELERY MOSAIC. *Phytopathology* 24: 695-725, illus.
- (31) WINGARD, S. A.
1928. HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS. *Jour. Agr. Research* 37: 127-153, illus.
- (32) ZAUMEYER, W. J.
1933. TRANSMISSIBILITY OF CERTAIN LEGUME-MOSAIC VIRUSES TO BEAN. (Abstract) *Phytopathology* 23: 39.
- (33) ——— and WADE, B. L.
1933. MOSAIC DISEASES AFFECTING DIFFERENT LEGUMES IN RELATION TO BEANS AND PEAS. (Phytopath. Note) *Phytopathology* 23: 562-564.

THE VITAMIN A, B, C, D, AND G CONTENT OF THE OUTER GREEN LEAVES AND THE INNER BLEACHED LEAVES OF ICEBERG LETTUCE¹

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INTRODUCTION

The association of vitamin A with greenness of plants has been a subject of much interest almost since the discovery of this vitamin. In 1925, studies were begun in the Bureau of Home Economics to determine the vitamin A content of the outer green leaves and the inner bleached leaves of Iceberg lettuce, a variety of *Lactuca sativa*. While the results of this study were being prepared for publication the report of Dye, Medlock, and Crist (3)² on the association of vitamin A with greenness in plant tissues appeared. Publication was therefore delayed, and the studies were extended to include vitamins B, C, D, and G. The present report covers all of these studies, although some of the evidence obtained merely corroborates the findings reported by other investigators.

The tests were completed before the International vitamin standards of reference became available for general use. Inasmuch as the purpose of the study was to determine the relative vitamin potency of the green and the bleached leaves, the results are still valid even though they cannot be interpreted in terms of International units.

MATERIAL

All tests were made with Iceberg lettuce purchased on the Washington retail market. Most of it was grown in California. The inner bleached leaves were from the head as it is offered for sale by the retailer, care being taken to discard all leaves showing an appreciable amount of greenness. The outer green leaves were those generally trimmed from the head before it is sold. These were secured regularly by a special arrangement with the grocer. As soon as the lettuce was received it was carefully washed and stored in a covered receptacle in the refrigerator until it was to be used. The excess water was patted off with a dry towel before the portions for the rats were weighed. The thick part of the stem at the base of the leaves was discarded.

PROCEDURE AND RESULTS

VITAMIN A

The vitamin A tests were made by the Sherman-Munsell technique (7). The basal diet was irradiated to supply vitamin D. The green leaves were fed at levels of 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.8 g per

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² Reference is made by number (italic) to Literature Cited, p. 1046.

rat 6 times per week, and the bleached leaves at levels of 0.4, 0.8, and 1.2 g. The results, given in table 1, indicate that the green leaves contained about 34.5 Sherman units per gram while the bleached leaves contained only 1 unit per gram. Kramer and her associates (5) in 1929 reported results indicating that the green leaves were 30 or more times as rich as the bleached leaves.

TABLE 1.—*Vitamin A content of Iceberg lettuce*

Leaves fed	Quantity fed per rat 6 times per week	Rats used in test	Average gain in weight of rats during 8 weeks	Standard error of mean gain of rats	Estimated quantity of lettuce to give 25 g gain in 8 weeks	Vitamin A units (Sherman) per gram of lettuce
	<i>Grams</i>	<i>Number</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	
Outer green.....	0.0125	7	8.7	2.7	0.029	34.5
	.025	11	22.1	3.7		
	.05	6	39.5	5.6		
	.1	7	55.3	9.0		
	.2	6	73.2	8.6		
	.8	6	66.0	12.6		
Inner bleached.....	.4	7	5.2	7.7	1.0	1.0
	.8	14	22.7	3.6		
	1.2	13	27.9	3.3		

¹ Average of 6 surviving animals.

VITAMIN B

Tests for vitamin B were made according to the method described by Chase and Sherman (2). Rats, weaned at the age of 4 weeks, were given the vitamin-B-free diet. At the end of 2 weeks they were placed in individual cages and allotted portions of lettuce as their only source of vitamin B (B₁).

A short time before these tests were begun a new colony of rats had been established in the laboratory from a few animals obtained from the Wistar Institute. This colony was maintained on the same stock diet as the regular colony established some 10 years ago from which animals are drawn for vitamin B and vitamin G tests. One assay was made with young rats from this new colony (B) in order to check their behavior when under test. The other assay was made with animals from the regular stock colony (A). The results of both assays are shown in table 2.

The green leaves were fed at levels of 2, 4, and 6 g and the bleached leaves at levels of 1, 2, 4, and 6 g in the test with rats from colony A. In the second series with rats from colony B, both the green and bleached leaves were fed at levels of 2, 4, and 6 g. With one exception where only one female was used, each group of animals on the different levels of lettuce contained the same number of males and females. Furthermore, for the most part each individual of a group on any one level of a given kind of lettuce was paired with a litter mate of the same sex in the groups on the other levels. These precautions are essential to the derivation of Sherman units, and also make more valid the comparison between the results obtained with the green and bleached leaves.

TABLE 2.—Vitamin B content of Iceberg lettuce

Description of leaves fed	Tests with rats from colony A						Tests with rats from colony B					
	Quantity fed per rat 6 times per week	Rats used in test ¹	Average gain (+) or loss (-) in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin B units (Sherman) per gram of lettuce	Ratio of vitamin B content of green to bleached leaves	Quantity fed per rat 6 times per week	Rats used in test ¹	Average gain (+) or loss (-) in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin B units (Sherman) per gram of lettuce	Ratio of vitamin B content of green to bleached leaves
	<i>Grams</i>	<i>Number</i>	<i>Grams</i>				<i>Grams</i>	<i>Number</i>	<i>Grams</i>			
Outer green.....	2	5 M 8 F	-11.1	3.8	0.24	0.80	2	8 M 8 F	-2.4	2.8	0.27	0.69
	4	8 M 5 F	+21.5	2.4			4	8 M 8 F	+30.8	1.8		
	6	2 M 2 F	+50.5	-----			6	8 M 8 F	+56.4	3.3		
	1	5 M 5 F	-11.9	2.6			2	8 M 8 F	+14.4	3.1		
	2	5 M 5 F	+2.8	3.2			4	8 M 8 F	+52.6	3.4		
	4	5 M 5 F	+36.1	3.0			6	8 M 8 F	+75.1	5.0		
Inner bleached....	6	1 F	+51.0	-----	.30						.39	

¹ M=male and F=female.² Average of 8 surviving animals.

In the test with animals from colony A the ratio of potency of green leaves to bleached was 0.80, while in the second test it was 0.69. This is a rather close agreement considering the variability of results obtained by the biological method. In 1931 Kohman, Eddy, and Gurin (4) reported findings indicating that the outer green leaves were appreciably richer in the vitamin B complex than the inner bleached leaves.

VITAMIN C

Tests for vitamin C content, conducted according to the technique of Sherman, LaMer, and Campbell (6), were made at two different times. In the early tests the levels of lettuce fed to the guinea pigs were 6, 12, 15, 18, and 21 g per day. The results indicated that 21 g might be near the minimum protective dose, but the limited data obtained did not warrant forming definite conclusions. The assays have, therefore, been repeated recently with daily feedings of 15, 18, and 21 g. The results of the tests at these levels are given in table 3. All animals survived the full 90 days of the test period. The minimum protective dose of the green leaves seems to be slightly more than 21 g and of the bleached leaves about 21 g. Kohman, Eddy, and Gurin (4) have reported the minimum protective dose as between 15 and 25 g. They state that there is very little difference between the green and the bleached leaves.

TABLE 3.—Vitamin C content of Iceberg lettuce

Quantity of lettuce fed per guinea pig 6 times per week (grams)	Tests with outer green leaves				Tests with inner bleached leaves			
	Guinea pig no. ¹	Total gain in weight of guinea pig during 90 days	Degree of scurvy	Scurvy score	Guinea pig no. ¹	Total gain in weight of guinea pig during 90 days	Degree of scurvy	Scurvy score
15.....	B23 M.....	Grams 220	Trace.....	3.5	B26 M.....	Grams 348	None.....	0
	B31 M.....	260	Mild.....	5.5	B33 M.....	226	Moderate.....	11.5
	42 M.....	25	do.....	8	B34 M.....	171	do.....	3.5
	53 M.....	72	Moderate.....	2	43 M.....	196	Trace.....	2.5
	356 M.....	366	Trace.....	3	355 F.....	120	do.....	3
	377 F.....	151	Moderate.....	7	366 M.....	145	do.....	2
	383 M.....	366	Trace.....	2	375 M.....	204	Mild.....	5
					382 F.....	179	None.....	0
	Average.....	208.6		4.4		198.6		3.4
	55 M.....	-23	Moderate.....	13	57 F.....	191	Mild.....	1.5
	56 M.....	118	Mild.....	2	83 M.....	264		7
	59 M.....	135	do.....	1.5				
	80 M.....	108	Trace.....	3				
	84 M.....	369		2				
	357 F.....	258	Trace.....	2	354 M.....	363	Trace.....	2
18.....	365 M.....	20	Mild.....	7	373 F.....	288	do.....	1
	378 F.....	279	Trace.....	4	380 F.....	211	do.....	1
	379 M.....	345	do.....	1				
	Average.....	178.2		3.9		263.4		2.5
	82 M.....	349	Moderate.....	0.5	81 F.....	267		2.5
21.....	86 M.....	316		0	85 M.....	246		0
	364 F.....	53	Moderate.....	9	89 F.....	268		0
	371 M.....	222	Trace.....	3	368 F.....	155	None.....	0
					372 M.....	341	Trace.....	2
	Average.....	235		3.1		255.4		.9

¹ M = male and F = female.

VITAMIN D

The vitamin D tests were made by the usual line-test technique. Rats 28 days old were placed on a Steenbock yellow-corn low-phosphorus ration until they developed severe rickets. They were then given nine daily feedings of lettuce. At the end of the ninth day they were killed and the line test was made. The lettuce was fed at levels of 2, 3, and 4 g and one rat received 5 g. No healing was produced even at the 5-g level. This would seem to be sufficient evidence that lettuce does not contain detectable amounts of vitamin D.

VITAMIN G

The vitamin G tests were made according to the technique worked out in this laboratory. It is practically the same as the method described by Bourquin and Sherman (1), except that an alcoholic extract of rice polishings is used as a source of vitamin B. In these tests as in the vitamin B tests, two assays were made, one with animals from colony A and the other with animals from colony B. In the first test the outer green leaves were fed at levels of 0.5, 1, and 2 g and the inner bleached leaves at levels of 1, 2, and 4 g. In the tests with animals from colony B both kinds of leaves were fed at levels of 2, 4, and 6 g. As in the tests for vitamin B, care was taken

to have equal numbers of males and females in each group of animals on the different levels of lettuce, and as far as possible to have the groups fed one kind of lettuce contain a representative of the same sex from each litter of test animals used.

In these vitamin G assays very different results (table 4) were obtained with the rats from the two colonies. In the first test made during the late summer and fall the green leaves assayed 0.46 Sherman unit per gram and the bleached leaves 0.24 unit per gram. In the second test made in spring and late summer the green leaves gave a value of 1.18 units and the bleached leaves 0.67 unit per gram. The ratios between green and bleached for the two tests were respectively 1.91 and 1.76. Thus the relative potency shown by the results of the two tests was very similar although the absolute values were very different. More recent work in this laboratory has corroborated this variable behavior of rats from different colonies when they are confined on a vitamin-G-deficient diet. Of course there is a possibility that the differences may have been due to seasonal variation in the vitamin content of the lettuce although there are no data to substantiate this.

TABLE 4.—Vitamin G content of Iceberg lettuce

Description of leaves fed	Tests with rats from colony A, late summer and fall						Tests with rats from colony B, spring and late summer							
	Quantity fed per rat 6 times per week	Rats used in test 1		Average gain in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin G units (Sherman) per gram of lettuce	Ratio of vitamin G content of green to bleached leaves	Quantity fed per rat 6 times per week	Rats used in test 1		Average gain in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin G units (Sherman) per gram of lettuce	Ratio of vitamin G content of green to bleached leaves
	Grams	Number	Grams					Grams	Number	Grams				
Outer green-----	0.5	5 M 5 F	8.4	3.2	0.46	1.91		2	5 M 5 F	44.1	3	1.18	0.67	1.76
	1	5 M 5 F	18.5	6.1				4	5 M 5 F	62.9	2.1			
	2	5 M 5 F	23.7	2.9				6	5 M 5 F	80.4	3.7			
Inner bleached-----	1	5 M 5 F	13.6	3.2	.24			2	5 M 5 F	29.9	3	.67		
	2	5 M 5 F	19.4	5.4				4	5 M 5 F	40.7	3.1			
	4	5 M 5 F	24.4	3.9				6	5 M 5 F	47.9	3.4			

¹ M=male and F=female.

SUMMARY

The outer green leaves and the inner bleached leaves of Iceberg lettuce (*Lactuca sativa*) were assayed for vitamins A, B, C, D, and G.

The vitamin A content was derived as 34.5 units (Sherman) per gram in the green and 1 in the bleached leaves.

Two assays for vitamin B made with test animals from two different colonies showed values of 0.24 and 0.27 unit (Sherman) per gram for the green and 0.30 and 0.39 for the bleached leaves.

The vitamin C test indicated that the minimum protective level for the green leaves was slightly more than 21 g. Very nearly complete protection was obtained with 21 g of the bleached leaves.

Vitamin D could not be detected in either the green or the bleached leaves, although quantities as high as 5 g per day were fed to the test animals.

Vitamin G assays, likewise made with animals from two colonies, showed in the one case 0.46 unit (Sherman) per gram for green leaves and 0.24 for the bleached. In the other test the values were 1.18 and 0.67 respectively. The ratios of potency of green to bleached leaves in the two tests, however, were 1.91 and 1.76.

LITERATURE CITED

- (1) BOURQUIN, A., and SHERMAN, H. C.
1931. QUANTITATIVE DETERMINATION OF VITAMIN G(B₂). *Jour. Amer. Chem. Soc.* 53: 3501-3505, illus.
- (2) CHASE, E. F., and SHERMAN, H. C.
1931. A QUANTITATIVE STUDY OF THE DETERMINATION OF THE ANTI-NEURITIC VITAMIN B. *Jour. Amer. Chem. Soc.* 53: 3506-3510, illus.
- (3) DYE, M., MEDLOCK, O. C., and CRIST, J. W.
1927. THE ASSOCIATION OF VITAMIN A WITH GREENNESS IN PLANT TISSUE. I. THE RELATIVE VITAMIN A CONTENT OF HEAD AND LEAF LETTUCE. *Jour. Biol. Chem.* 74: 95-106, illus.
- (4) KOHMAN, E. F., EDDY, W. H., and GURIN, C. Z.
1931. VITAMINS IN CANNED FOODS. X THE VITAMIN CONTENT OF SOME COMMON VEGETABLES. *Indus. and Engin. Chem.* 23: 808-811, illus.
- (5) KRAMER, M. M., BOEHM, G., and WILLIAMS, R. E.
1929. VITAMIN A CONTENT OF THE GREEN AND WHITE LEAVES OF MARKET HEAD LETTUCE. *Jour. Home Econ.* 21: 679-680.
- (6) SHERMAN, H. C., LA MER, V. K., and CAMPBELL, H. L.
1922. THE QUANTITATIVE DETERMINATION OF THE ANTISCORBUTIC VITAMIN (VITAMIN C). *Jour. Amer. Chem. Soc.* 44: 165-172, illus.
- (7) ——— and MUNSELL, H. E.
1925. THE QUANTITATIVE DETERMINATION OF VITAMIN A. *Jour. Amer. Chem. Soc.* 47: 1639-1646, illus.

INTERSPECIFIC HYBRIDIZATION IN *GOSSYPIMUM* AND THE MEIOTIC BEHAVIOR OF F_1 PLANTS¹

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INTRODUCTION

The present study deals with F_1 hybrids between and within the following five morphologically distinct groups of *Gossypium*: (1) Cultivated American species; (2) wild American species; (3) cultivated Asiatic species; (4) a wild Australian species, *G. sturtii* F. Muell.; and (5) a wild American plant, *Thurberia thespesioides* A. Gray, possibly congeneric with *Gossypium*. Each of these five groups, with one exception, has the haploid chromosome number 13; in the cultivated American group, the haploid chromosome number is 26. The scientific and economic importance of the groups is indicated by the rapidly increasing amount of literature dealing with them.

Previous cytological studies of *Gossypium* have shown the relationships and possible origin of the species in several groups. Skovsted (23)² points out that Lawrence (18) suggests the possibility that Asiatic cottons are secondary polyploids. Lawrence's conclusions are based upon the chromosome association depicted by Denham (18, p. 367) and the complex inbreeding results obtained by Harland (18, p. 376). The occurrence of trisomes in a haploid cultivated American cotton, together with genetical data, led Skovsted to support Lawrence's assumption. Skovsted offers two alternative explanations of the origin of certain Asiatic species: (1) The doubling of chromosomes following the "crossing of two different but closely allied 7-chromosome species with the same 6-chromosome species" and (2) the doubling of a complement "composed of two similar sets of 6, and one chromosome appearing a third time."

During meiosis interspecific Asiatic hybrids have generally been reported to form 13 pairs of chromosomes and to exhibit normal cytological behavior (1, 4). Skovsted (23), however, records a decrease in the number of chiasmata in each pair of chromosomes and a very slight irregularity in first anaphase distribution. In a triploid Asiatic hybrid Skovsted found that polysomes up to septivalents were formed. Skovsted's findings demonstrate that autosyndesis occurred between the chromosomes in a haploid set, and further support the assumption that Asiatic species are of polyploid nature.

Davie (3) suggests that the chromosome complement of *Gossypium herbaceum* L. is derived from the basic number 7. In support of his assumption he points out that two of the somatic chromosomes are longer than the rest and that secondary chromosome association and

¹ Received for publication July 12, 1935; issued February 1936.

² Reference is made by number (italic) to Literature Cited, p. 1089.

quadrivalent chromosomes occasionally occur during meiosis. He states (3, p. 63):

The origin of the genus *Gossypium* appears to have involved tetraploidy on a basis of 7 followed by fusion of two pairs of chromosomes into one pair, thus giving $n=13$ as the fundamental number for the genus. From those so-called "diploid Asiatic cottons", which are really modified tetraploids, have arisen the so-called "tetraploid American cottons", which are phylogenetically modified octoploids. The "tetraploid" American cottons have not necessarily a common ancestor. Amphidiploidy has probably occurred in the different hybrids which result from crosses between various related ancestral diploid species. In this way the American cottons, with $2n=52$, may have arisen from separate ancestors.

Baranov (1) found chromosome distinctions within the somatic complement of both Asiatic and cultivated American species. Skovsted (24) reports that half of the chromosomes of the cultivated American species are small and the other half larger. The Asiatic and the Australian species have chromosomes comparable in size to the larger ones occurring in the cultivated American species, and the small chromosomes of the latter species are of the same size as those found in wild American species. Skovsted further records that 2 of the chromosomes of the cultivated American species and 4 of those of the Asiatic species have satellites.

The double chromosome number found in cultivated American species suggested to Longley (19) a duplication of the chromosomes of an ancestral type. Longley believes that his assumption is supported by the breeding results obtained in Asiatic and cultivated American species (cf. Matsuura, 20). Longley also suggests that the high chromosome number of the cultivated American species possibly arose through hybridization. Five species, *Gossypium stockii* Mast. (wild Asiatic), *G. sturtii* (Australian), *G. davidsonii* Kellogg (wild American), *G. harknessii* Brandeg. (wild American), and *Thurberia thespesioides*, are listed by Longley as probably most closely representing the ancestral type of species involved in the origin of cultivated American species. The occurrence of quadrivalent chromosomes and apparently of secondarily paired bivalents in cultivated American species led the writer (27) to support Longley's suggestion.

In general, interspecific cultivated American-Asiatic hybrids have been reported to form 13 univalent and 13 bivalent chromosomes during the first meiotic division (1, 21). Skovsted (24), however, records the frequent occurrence of polyvalents, with a conjugation as great as hexavalents. He also points out that in a triploid Asiatic-cultivated American hybrid ($3n=52$) the meiotic chromosome conjugation is the same as that found in the triploid Asiatic hybrid, except for the addition of 13 univalent chromosomes. This chromosome behavior and the fact that at least 13 univalents are always present in both $2n$ and $3n$ hybrids between cultivated American and cultivated Asiatic species led Skovsted to conclude that the pairing in the $2n$ hybrid was due to allosyndesis.

On the basis of the two groups of chromosomes found in the cultivated American species, together with the type of pairing (allosyndesis) in cultivated American-Asiatic hybrids, Skovsted concludes that the cultivated American species are allopolyploids. He suggests that one of the parental species was an Asiatic cotton or closely allied type, while the other was probably a wild American species characterized by 13 smaller chromosomes.

In a recent note the writer (28) set forth the essential cytological features of several interspecific hybrids in cotton. Since the cytological behavior of these hybrids bears upon the relationships disclosed in the preceding paragraphs, it appears desirable to give a more detailed account of their behavior. Additional data from new hybrids give further indications of phylogenetic relationships.

PARENTAL SPECIES AND VARIETIES

The plants used for this study were from the collection of living plants of the tribe Hibisceae of the family Malvaceae at the Rubidoux Laboratory, Riverside, Calif. Although numerous additional species and varieties were cross-pollinated, only the parents of crosses that yielded F_1 plants are listed.³

Cultivated American group ($n=26$)

Gossypium hirsutum L. (723)⁴

commercial vars. Acala (W 24) and Rowden (W 12)

G. contextum Cook and Hubbard (542)

G. punctatum Schum. and Thon. (437)

G. schollii Watt (672)

G. barbadense L. (W 5)

commercial var. Pima (W 23)

Wild American group ($n=13$)

G. davidsonii Kellogg (101)

G. harknessii Braud. (861)

G. armourianum Kearney (867)

Cultivated Asiatic group ($n=13$)

G. arboreum L.

Taxonomic vars. *sanguineum* Watt (787) and *neglectum* Watt (785)

G. africanum Watt (419)

G. herbaceum L. (743)

Wild Australian species ($n=13$)

G. sturtii F. Muell. (632)

Wild Arizona species ($n=13$)

Thurberia thespesioides A. Gray (112)

The species and varieties listed are described by Watt (26), Oakley (22), Cook and Hubbard (2), Harland (10), and Kearney (12, 15, 16). Their meiotic chromosome behavior is described by Webber (27) and Longley (19).

INTERSPECIFIC HYBRIDIZATION

METHODS

In general, the method employed in making cross-pollinations was similar to that described by Kearney and Porter (17). The flowers of the pistillate parent were emasculated the evening before anthesis and were enclosed in bags. The same evening, the flowers of the staminate parent were prevented from opening by means of a spirally twisted wire. The next morning, immediately after anthesis, cross-pollinations were made. In the majority of cases the latter process was accomplished before untreated flowers of the staminate parent were completely expanded. Immediately after pollination the protective bags were replaced. These bags were removed approximately 30 hours later, at which time the stigma is apparently no longer receptive.

³ In view of the confusion prevailing in the taxonomy of the cultivated cottons, it is not assumed that all of the species listed here are valid.

⁴ The numbers in parentheses following the names of the species and varieties are those under which the seeds are cataloged by the Division of Cotton and Other Fiber Crops and Diseases, as explained by Webber (27).

In order to increase the percentage of successful cross-pollinations, several cotton breeders have developed special methods. These methods mainly involve the application of chemical solutions to the stigma or the treatment of the stigma with extracts of pollen, stigmas, and petals. The writer has tried several of the most promising methods and in general found that the application of chemicals or extracts to the stigma did not materially increase the percentage of successful cross-pollinations. The reported success obtained by such methods (5) is undoubtedly partially due to the development of parthenocarpic capsules. In cross-pollinations involving certain species and forms of cotton, the development of capsules which prove to be nothing more than empty shells is quite characteristic.

Doak (6) describes a method of emasculating cotton flowers which has proved very successful. It involves the splitting of the staminal column with the finger nail and pulling off the entire corolla and androecium in a single piece. This method leaves the pistil completely exposed and prevents it from being ruptured during normal shedding of the corolla. The method undoubtedly makes the stigma receptive to pollen over a longer period and prolongs the period of pollen-tube growth. The method was first suggested by Zaitzev (29) and has been successfully employed by the writer for several years.

Data given in table 1 indicate that the percentage of successful cross-pollinations depends on the time of day when the pollinations are made. The higher percentage of successful cross-pollinations obtained between 8:30 and 10:30 a. m. was undoubtedly due to the use of fresh pollen. In certain cotton species Kearney (13) has shown that the viability of the pollen "increases rapidly between 8 and 9 a. m., and shows a gradual decline after midday." Other data given in table 1 show an increase in the percentage of successful cross-pollinations in late summer, which may be due either to weather changes or to a physiological change within the pistillate parent.

TABLE 1.—Results of cross-pollinations in cotton made (1) in the morning and in the afternoon and (2) early and late in the season

Time of cross-pollination	Total cross-pollinations	Successful cross-pollinations		Time of cross-pollination	Total cross-pollinations	Successful cross-pollinations	
	Number	Number	Percent		Number	Number	Percent
8:30-10 30 a m	215	28	13.03	May 15-July 31----	186	17	9.14
1-3 p m	100	8	8.00	Aug. 1-Sept. 30----	129	19	14.73

In table 2 is given the number of cross-pollinations made within or among the five groups of species of *Gossypium*. In each of the combinations, except *Thurberia thespesioides* × *Gossypium sturtii*, several species of one or both groups were involved. The table also gives the percentage of successful cross-pollinations, the number of seeds obtained, and the percentage of germination of the seeds of cross-pollinated F₁ plants.

Table 2 shows that in most of the combinations cross-pollination was fairly successful. The correlation between the degree of relationship of the species involved and the success of cross-pollination is discussed later.

TABLE 2.—Results of cross-pollinations within and among the five groups of species of *Gossypium*

Combination	Total cross-pollinations	Successful cross-pollinations	Seeds from cross-pollinations	Germination ¹ of seeds from cross-pollinations
	Number	Percent	Number	Percent
Cultivated American × cultivated American.....	16	50.00	164	90
Cultivated American × <i>G. sturtii</i>	11	18.18	38	60
Wild American × <i>G. sturtii</i>	20	30.00	63	58
Asiatic × <i>G. sturtii</i>	25	12.00	10	(²)
Cultivated American × wild American.....	27	18.52	56	70
Asiatic × wild American.....	17	5.89	5	(²)
Wild American × wild American.....	5	20.00	13	80
Asiatic × Asiatic.....	36	11.11	49	70
Cultivated American × Asiatic.....	125	3.19	6	(³)
Cultivated American × <i>Thurberia thespesioides</i>	23	4.35	2	100
<i>T. thespesioides</i> × <i>G. sturtii</i>	10	10.00	11	50
Total or average.....	315	11.43	417	41

¹ Except in cases otherwise noted, this percentage is based upon 10 seeds placed between moist cotton at 28° C.

² 4 seeds tested; no germination.

³ 2 seeds tested; no germination.

⁴ 1 seed tested; no germination.

DESCRIPTION OF HYBRIDS

The following interspecific hybrids have been grown to maturity:

Hybrids within the cultivated American group ($n=26$):

W 47.—*Gossypium hirsutum* (W 24) × *G. punctatum* (437)

W 50.—*G. hirsutum* (723) × *G. barbadense* (W 23)

W 51.—*G. contextum* (542) × *G. schottii* (672)

W 46 and W 49.—*G. barbadense* (W 23) × *G. schottii* (672)

W 54 and W 53.—*G. barbadense* (W 5) × *G. punctatum* (437)

Hybrids within the cultivated Asiatic group ($n=13$):

W 45.—*G. arboreum* var. *sanguineum* (787) × *G. africanum* (419)

W 48.—*G. herbaceum* (743) × *G. arboreum* var. *neglectum* (785)

Hybrids within the wild American group ($n=13$):

W 41.—*G. harknessii* (861) × *G. armourianum* (867)

Hybrids between the cultivated American group ($n=26$) and the wild American group ($n=13$):

W 43 and W 34.—*G. hirsutum* (W 24) × *G. armourianum* (867)

W 56.—*G. contextum* (542) × *G. armourianum* (867)

W 38 and W 36.—*G. barbadense* (W 5) × *G. harknessii* (861)

Hybrids between the cultivated American group ($n=26$) and the wild Australian species ($n=13$):

W 57 and W 33.—*G. barbadense* (W 5) × *G. sturtii* (632)

Hybrids between the wild American group ($n=13$) and the wild Australian species ($n=13$):

W 39, W 52, and W 35.—*G. sturtii* (632) × *G. harknessii* (861)

W 40.—*G. sturtii* (632) × *G. armourianum* (867)

W 59.—*G. davidsonii* (101) × *G. sturtii* (632)

Hybrids between *Thurberia* ($n=13$) and the wild Australian species ($n=13$):

W 58.—*Thurberia thespesioides* (112) × *Gossypium sturtii* (632)

The following interspecific hybrids did not reach maturity:

Hybrids between the cultivated American group ($n=26$) and *Thurberia* ($n=13$):

W 44.—*Gossypium hirsutum* (W 12) × *Thurberia thespesioides* (112)

With the exception of *Gossypium hirsutum* × *Thurberia thespesioides* all of these F_1 hybrids flowered freely. The hybrids between species within a group are self-fertile and produce seed when fecundated with pollen of either parental species; also, both parental species are fertile

with the hybrid pollen. Such hybrids exhibit considerable hybrid vigor. The hybrids between species of different groups, on the contrary, are perfectly sterile and produce no seed when backcrossed to either parent. The parents also are sterile to the hybrid pollen. With the exception of *G. hirsutum* \times *T. thespesioides* all of these hybrids are exceedingly vigorous.

In several instances, the individuals of a hybrid between a pair of species exhibited variation in certain characters. Since other hybrids involving one or the other of the same parental species exhibited similar variations, it may be supposed that the common parent was heterozygous. In order to prevent repetition and complications in the following descriptions, such variations and numerous minor characteristics of the hybrids are not described.⁵

Gossypium hirsutum \times *punctatum*.—Plants *hirsutum*-like in all characters, sparsely hirsute on the young stems and petioles. Petals very pale yellow, anthers pale yellow as in *punctatum*. Bolls with 4 to 5 locules, large, nearly pointless, with or without very short apical furrows. Growth habit somewhat prostrate.

Gossypium hirsutum \times *barbadense*.—Typical of this combination in F₁. Petals large, *martius* yellow, with rather faint, striate spots. Pollen empire yellow. Bolls large, somewhat pointed, smooth.

Gossypium contextum \times *schottii*.—Stems, petioles, leaf veins, and involucre very dark purple. Leaf blades deeply and narrowly lobed. Petals spotless, lighter than *martius* yellow. Anthers approximately empire yellow. Bolls with four locules, somewhat taper-pointed, very smooth, with short but pronounced apical furrows.

Gossypium barbadense \times *schottii*.—Plants glabrous or nearly so, except on very young parts. Stems dark purple. Leaf blades deeply and narrowly five-lobed. Calyx with merely undulate margin and many oil glands. Petals pale green-yellow, distinctly spotted. Pollen between empire yellow and lemon-chrome. Bolls mostly with four locules, pointed, rather deeply pitted, with short but pronounced apical furrows.

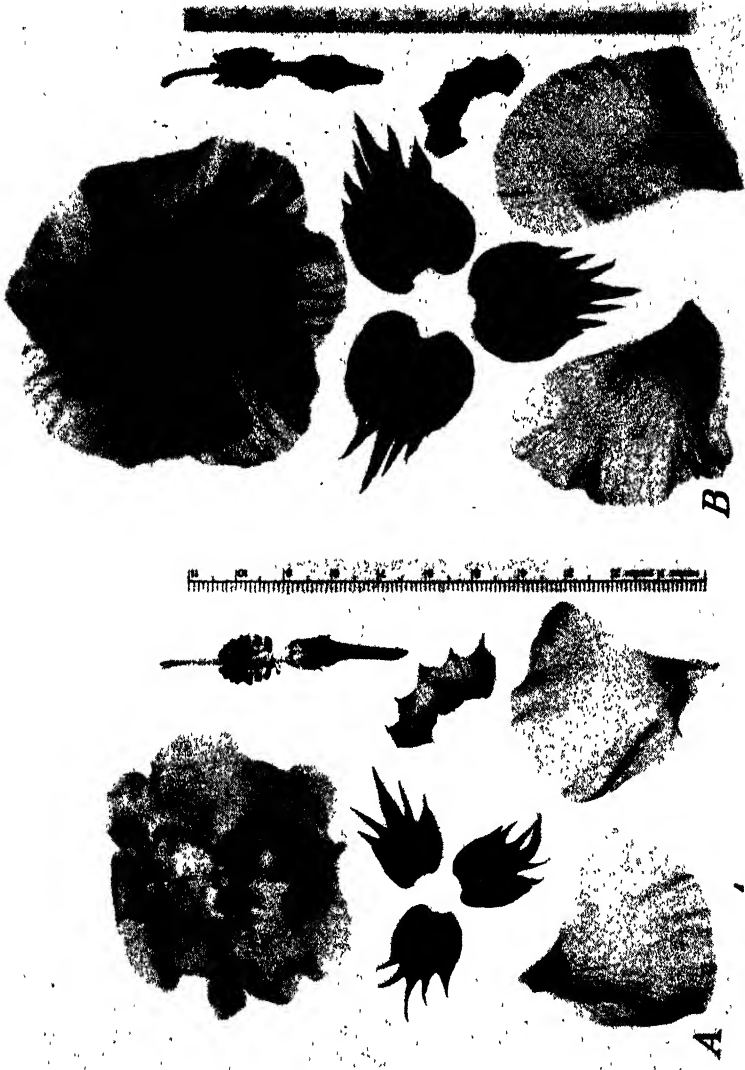
Gossypium barbadense \times *punctatum*.—Plants much as in *G. hirsutum* \times *barbadense* F₁, nearly glabrous except on the very young parts. Petals approximately *martius* yellow, with rather faint striate spots. Pollen near empire yellow. Bolls with 3 to 5 locules, their surface smooth and light colored.

Gossypium arboreum var. *sanguineum* \times *africanum*.—The characters of both parents are about equally represented in this hybrid. Young stems, petioles, and peduncles very hirsute. Older stems dark brown. Leaf blade more like that of *sanguineum*, deeply lobed, with relatively narrow acuminate lobes. Involucels like *sanguineum* in having bractlets of more triangular shape but deeply lacinate with setose-tipped teeth as in *africanum*. Petals ruffled on the outer edge, nearly as large as in *africanum*, approaching *sanguineum* in color (pomegranate-purple), but shading to yellowish around the spot rather than, as in *sanguineum*, to whitish. Spot intense bordeaux color. Anthers near xanthine-orange. Bolls mostly with three locules, oblong-ovoid, short-pointed, smooth, reddish where exposed.

Gossypium herbaceum \times *arboreum* var. *neglectum*.—Older stems nearly black; twigs, petioles, and peduncles hirsute. Leaf blades much nearer *neglectum* in shape. Involucel resembling *neglectum* in its more triangular bractlets with slenderer, more setose teeth, the bractlets connate near base. Calyx like *herbaceum* in its undulate or very short-dentate margin, the teeth deltoid and not, as in *neglectum*, setose-tipped. Petals approaching *neglectum* in size and shape and in the larger size of the very intense spot; between *martius* yellow and *picric* yellow, the spot between carmine and ox-blood red. Anthers light cadmium. Bolls mostly with four locules, short-ovoid, very plump, abruptly pointed, with long, deep apical furrows, red where exposed.

Gossypium harknessii \times *armourianum*.—Plants more open and with more ascending branches than in either parent, obscurely puberulent on the very young parts, soon glabrous. Twigs light mahogany colored, older bark light

⁵ The following descriptive notes were supplied mainly by T. H. Kearney. The colors were determined by comparison with the standards in the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.



Typical F₁ flowers and flower parts: A, *Gossypium hirsutum* × *armourianum*. B, *G. barbadense* × *harknessii*.



Gossypium barbadense \times *strutii*. Typical F₁ flower and flower parts. The petals are rose red in color; in the parents they are yellow and pale mauve respectively.

brown. Leaf blades grayish green (yellowish in *harknessii*, deeper green in *armourianum*), less shiny above than in *armourianum*, nearer *harknessii* in size and shape, entire to very shallowly three-lobed with rounded lobes, broadly ovate, obtuse or acutish, rather deeply cordate. Involucel light green, resembling that of *harknessii* in its large, oblong-ovate bractlets, which are persistent until anthesis. Petals approaching those of *harknessii* in size and shape, pale viridine yellow. Bolls mostly with three locules, broadly ovoid.

Gossypium hirsutum × *armourianum*.—Plants glabrous except on the very young parts, large, woody, many-stemmed but open, the branches wide-spreading, with long internodes, the branchlets conspicuously zigzag. Leaf blades light green with a small brown pulvinus, mostly shallowly three-lobed. Involucel persistent after anthesis; bractlets separate, with a few long teeth. Calyx dentate, sometimes with deltoid-setose teeth nearly as long as the height of the undivided portion and having very numerous oil glands. Petals pale green-yellow or martius yellow, reddish on the exposed edges; spot when present^a faint and striate to rather intensely pomegranate-purple. Filaments colorless, or the lowest purple. Anthers with or without red color on the connective. Pollen light cadmium. Exserted portion of the pistil very long. Plate 1, A, shows characteristic flower parts of this hybrid.

Gossypium contextum × *armourianum*.—Closely similar in all characters to *G. hirsutum* × *armourianum*.

Gossypium barbadense × *harknessii*.—The plants show a predominance of *barbadense* characters. Stems decidedly woody, twigs reddish brown. Petioles less densely puberulent than in *harknessii*, with longer hairs. Leaf blades *barbadense*-like but smaller, the larger ones deeply five-lobed, puberulent with short, stellate hairs, with a deeper-colored pulvinus than in *barbadense* and with a small deltoid or lanceolate nectary on the midvein toward base. Involucel large, showing no sign of falling at anthesis (a character of *harknessii*); bractlets deeply lacinate with broad teeth, separate or nearly so. Calyx ciliate, with undulate margin and numerous black oil glands. Petals large, pale green-yellow in W 36, between martius yellow and picric yellow in W 38, the spots large but rather faint. Filaments purplish. Anthers light cadmium. Pistil with the length of the exserted portion equaling or surpassing that of the stamiferous portion of the column. Bolls (5 weeks old, parthenocarpic) resembling those of *harknessii* in shape and in the reddish-brown oil glands. A characteristic plant and characteristic flower parts of this hybrid are shown in figure 1 and in plate 1, B.

Gossypium barbadense × *sturtii*.—Plants glabrous or nearly so, resembling *sturtii* in their rather stiff habit, with long, nearly erect, vegetative branches. Fruiting branches with very long internodes. Young bark reddish, glaucous. Leaf blades not glaucous, varying on different plants from deeply and rather narrowly five-lobed to much more shallowly and broadly three-lobed, with large lanceolate or deltoid nectaries near the base of the midvein or of all three principal veins. Involucel glabrous, one-half to two-thirds as high as the corolla; bractlets separate to the base, more or less deeply lacinate with few or rather numerous subulate, setose-tipped teeth. Calyx ciliate, with numerous oil glands, sharply dentate, with deltoid-subulate teeth. Petals densely ciliate toward base, between Tyrian pink and rose colored (yellow in *barbadense*, pale mauve in *sturtii*) with very large, intense, feathered spots of *sturtii* character and varying from pomegranate-purple to bordeaux in color. Column very long and stamiferous nearly to its base, as in *sturtii*. Filaments purple. Anthers reddish. Pollen yellow. Pistil long-exserted, but the exserted portion much shorter than the stamiferous part of the column. Figure 2 shows a characteristic plant of *G. barbadense* × *sturtii*, and plate 2 shows characteristic flower parts.

Gossypium sturtii × *harknessii*.—Plants of intermediate habit, less stiff and erect than *sturtii*, but much less spreading than *harknessii*, more open than either parent, with numerous erect or ascending branches, nearly glabrous. Petioles obscurely puberulent toward apex. Leaf blades glaucescent, nearer *harknessii* in color, broadly deltoid, entire to rather deeply three-lobed, subcordate, acuminate, the lateral lobes rounded or acute, with a very small, slit-like nectary near the base of the midvein. Involucel more persistent than in *harknessii*, larger than in either parent, glabrous; bractlets widely separate, ovate or lance-ovate, setose-acuminate, narrowed at base, usually undulate or sparsely denticulate on the margin, but sometimes dentate with very few, short or long,

^a The *armourianum* parent was presumably heterozygous for this character.

subulate-setose teeth. Calyx very thin, obscurely ciliolate, dentate with short, deltoid, very acute teeth and with numerous oil glands. Petals of a rather dingy color between rose-pink and hellebore-red, with a broad, pinkish-buff stripe on the exposed edge, the spot large, much like that of *sturtii* in shape but less feathered, bright carmine. Column often very long, as in *sturtii*. Filaments much shorter than in *harknessii*; anthers between dragon-blood red and Etruscan red. Pollen pale yellow. Pistil with the exerted portion long but usually shorter than the column. Figure 3 shows a characteristic plant of *G. sturtii* \times *harknessii*.



FIGURE 1.—*Gossypium barbadense* \times *harknessii*. Typical F_1 plant.

Gossypium sturtii \times *armourianum*.—No open flowers have been produced. Plants minutely puberulent on the very young parts, soon glabrous, low, compact, with very numerous, ascending branches, much lower and more spreading than in *sturtii* but with fewer and less spreading branches than in *armourianum*. Leaf blades not glaucous and of about the same color as in *armourianum*, broadly ovate, subcordate, abruptly short-acuminate with a cartilaginous tip, usually with a small slitlike nectary near the base of the midvein. Involucel of intermediate size and character, more persistent than in *armourianum*; bractlets widely separate, narrowly lanceolate, entire, or occasionally with a minute tooth near apex, very sharply cartilaginous-acuminate. Calyx conspicuously dentate with deltoid-subulate teeth longer and slenderer than in *armourianum* and having numerous, conspicuous, prominent black oil glands.

Gossypium davidsonii \times *sturtii*.—Plants very open, with few ascending branches, obscurely puberulent toward the apex of the petiole, on the pulvinus, and near the base of the leaf veins, otherwise glabrous or very nearly so. Leaf blades glaucous but much less so than in *sturtii*, all entire or occasionally with a very short tooth, broadly ovate, short-acuminate, subcordate. Involucel nearly intermediate, obscurely ciliolate, otherwise glabrous; bractlets quite separate, oblong-ovate, with few short, deltoid, setose-tipped teeth. Calyx dentate with deltoid, obtuse to setose-acuminate teeth and with fairly numerous oil glands.



FIGURE 2.—*Gossypium barbadense* \times *sturtii*. Typical F_1 plant.

Corolla small. Petals pale rhodonite pink, the spot *sturtii*-like, pomegranate-purple. Column much as in *sturtii*, long, the stamiferous portion longer than the exerted part of the long-exserted pistil. Filaments short, purplish. Anthers pale orange-yellow, drying pinkish. Pollen pale yellow. Figure 4 shows a characteristic plant of *G. davidsonii* \times *sturtii*, and plate 3, A, shows characteristic flower parts.

Gossypium hirsutum \times *Thurberia thespesioides*.—The plants died soon after the seedling stage. Leaf blade much like that of *Thurberia* in shape, ovate-lanceolate, deeply and narrowly lobed.

Thurberia thespesioides \times *Gossypium sturtii*.—Plants much more open in habit than *G. sturtii*, with numerous nearly erect limbs; young branches somewhat

glaucous. Leaf blades green, slightly glaucous, much like those of *Thurberia* in shape, ovate-lanceolate and entire to deeply and narrowly three-lobed; lobes long-acuminate, setose at apex as in *G. sturtii*, with an elongate slitlike nectary very near the base of the midvein. Involucel with bractlets widely separate, as in both parents; bractlets lanceolate, intermediate in width but near *G. sturtii* in length, setose-tipped, often with 1 or 2 setose teeth. Calyx abruptly dentate with subulate-setose teeth. Petals approaching those of *G. sturtii* in size and shape, in color intermediate between white and pale amaranth-pink, fading to the latter color, the spot between pomegranate-purple and bordeaux, very like that of *G. sturtii*. Column much as in *G. sturtii*, elongate, with short filaments,

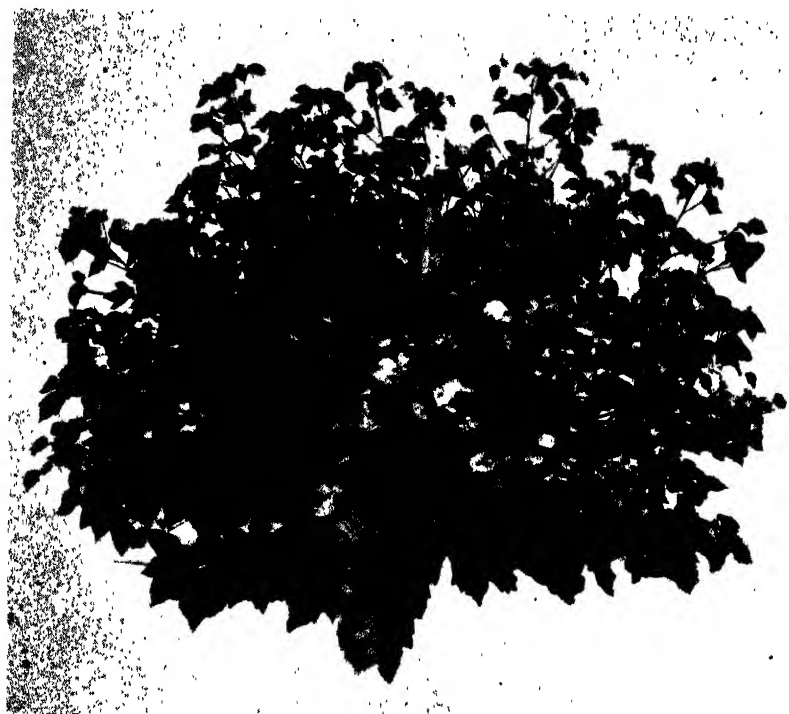


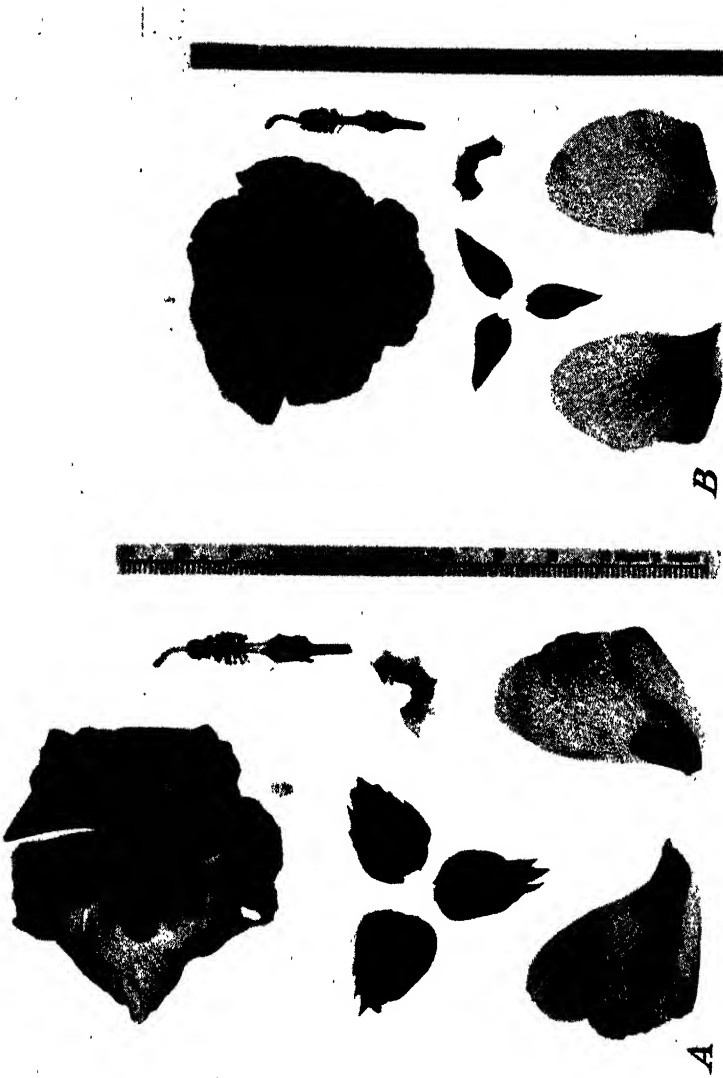
FIGURE 3.—*Gossypium sturtii* \times *harknessii*. Typical F_1 plant.

but with fewer stamens and these not extending so near the base of the column. Filaments purple. Anthers daphne pink. Pistil, with the exerted portion long but not equaling the column. Plate 3, B, shows characteristic flower parts of *T. thespesioides* \times *G. sturtii*.

CYTOLOGICAL TECHNIQUE

Cytological material was collected in full sunlight between May and September. In order to insure characteristic meiotic behavior, weekly checks were made with material from several individuals. Since these individuals continued throughout the season to exhibit the same cytological behavior, the data herein reported may be assumed to be characteristic of all representatives of a given combination.

The study was limited to pollen mother cells and sporads from anthers fixed in Carnoy's fluid 10 to 30 minutes, washed and preserved in alcohol, and crushed in iron-acetocarmine. The use of Carnoy's fluid and alcohol previous to crushing in acetocarmine is of advantage



Typical F₁ flowers and flower parts: A, *Gossypium daridsonii* × *sturtii*. B, *Thurberia thespesioides* × *G. sturtii*.

in that the extremely soft pollen mother cells of *Gossypium* are hardened and much of the matter obscuring the chromosomes is removed from them. Acetocarmine preparations have the distinct advantage of keeping pollen mother cells in a solution. Hence, the cells can be moved to allow vision at different angles and can be easily crushed to allow a more detailed study.



FIGURE 4.—*Gossypium davidsonii* \times *sturtii*. Typical F₁ plant.

All drawings were made at bench level with a camera lucida having a 1.5-mm objective (1.3 N. A.), an 18 \times compensating ocular, and a tube length of 160 mm, giving a magnification of 4,650 diameters.

MEIOTIC BEHAVIOR OF F₁ PLANTS

HYBRIDS BETWEEN CULTIVATED AMERICAN SPECIES (N 26 \times N 26)

The reduction divisions of the hybrids between species within the cultivated American group of cottons, listed on page 1051, are similar

to those of their parents (27). Although there are generally 26 bivalent chromosomes at first metaphase, occasionally 1 or 2, and rarely 3 quadrivalent chromosomes are formed. The bivalent partners are united at one or both ends by 1 or 2 or rarely 3 chiasmata. The total number of chiasmata is apparently somewhat smaller than in 26-paired species. Figure 5 depicts a characteristic first metaphase of *Gossypium barbadense* \times *schottii*.

During the first anaphase no irregularities occur and the homotypic divisions are nearly normal. Like the parental species, approximately 4 percent of the sporads contain microcytes.



FIGURE 5.—*Gossypium barbadense* \times *schottii*. Lateral view of first metaphase in a slightly crushed pollen mother cell, showing 26 bivalent chromosomes.

HYBRIDS BETWEEN ASIATIC SPECIES (N 13 \times N 13)

The meiotic divisions of *Gossypium arboreum* var. *sanguineum* \times *africanum* and of *G. herbaceum* \times *arboreum* var. *neglectum* also present a picture very similar to that of their parents (27). There are 13 bivalent chromosomes. At the first metaphase the total number of chiasmata is considerably smaller than that occurring in either parent and all chiasmata are strictly terminal. In *G. arboreum* var. *sanguineum* \times *africanum* the union between the partners of 2 bivalent chromosomes is apparently rather weak, and in several cases 2 univalent chromosomes have been observed. In the anaphase of the latter hybrid occasionally the univalents lag and rarely are left in the plasma. Although in both hybrids the majority of second metaphase plates contain 13 chromosomes, plates with 12 and 14 are not uncommon. Approximately 3 percent of the sporads of *G. herbaceum* \times *arboreum* var. *neglectum* and 6 percent of those of *G. arboreum* var. *sanguineum* \times *africanum* exhibit microcytes.

HYBRIDS BETWEEN WILD AMERICAN SPECIES (N 13 × N 13)

The meiotic divisions of *Gossypium harknessii* × *armourianum* are similar to those of both parents (27). During diakinesis and the first metaphase there are 13 pairs of chromosomes. Both the first and second divisions are regular and the tetrads are normal. Figure 6 depicts a characteristic first metaphase.

HYBRIDS BETWEEN CULTIVATED AMERICAN AND WILD AMERICAN SPECIES (N 26 × N 13)

During the first metaphase in *Gossypium hirsutum* × *armourianum* and *G. contextum* × *armourianum* there are usually 13 bivalent and 13 univalent chromosomes. Among 43 pollen mother cells of *G. hirsutum* × *armourianum*, 4 formed 1 quadrivalent, 11 bivalent, and 13 univalent chromosomes; and 1 formed 2 quadrivalent, 9 bivalent, and 13 univalent chromosomes. The bivalents form a well-organized equatorial plate which generally contains a few elongated univalents. The remaining univalents are more or less spherical and are scattered

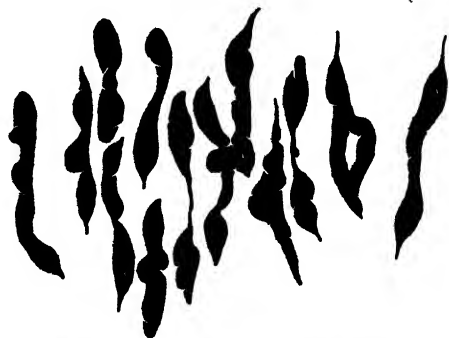


FIGURE 6.—*Gossypium harknessii* × *armourianum*. Profile view of first metaphase in a pollen mother cell, showing 13 bivalent chromosomes.

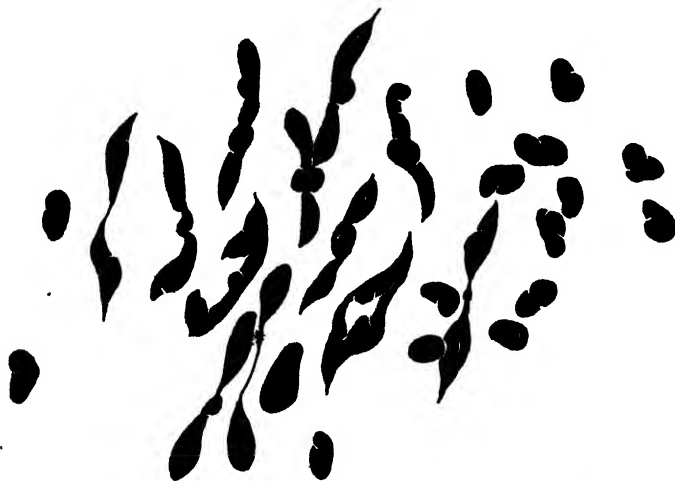


FIGURE 7.—*Gossypium hirsutum* × *armourianum*. Lateral view of first metaphase in a pollen mother cell, showing 12 bivalent and 15 univalent chromosomes.

over the achromatic figure. The majority of bivalent partners are united at one end by 1 or 2 terminal chiasmata. In some bivalents the union between partners is comparatively poor and rarely it is ineffectual. In the latter case 2, 4, or 6 additional univalent chromosomes occur. Figure 7, which illustrates the condition at the approach of the heterotypic anaphase, depicts 12 bivalent and 15 univalent chromosomes.

At the first anaphase the bivalent partners are distributed normally and the scattered univalents pass toward adjacent polar regions. The univalents in the equatorial region become laggards and elongate as though about to divide. They are later distributed at random toward the poles, but on account of their delayed movement they are frequently left in the plasma. Occasionally a univalent completes division and the resulting parts are left in the plasma or included in the daughter nuclei. Since the products of such division are of various sizes, the division is undoubtedly a fragmentation.

The homotypic divisions are fairly regular. There are usually two major and several diminutive achromatic figures. Although the majority of chromosomes divide normally, laggards are not uncommon. At the completion of meiosis highly abnormal sporads occur, which contain from 2 to 12 spores of various sizes.

The heterotypic divisions of *Gossypium barbadense* \times *harknessii* differ slightly from those of *G. hirsutum* \times *armourianum*. In no case have more than 13 univalent chromosomes been observed. In *G. barbadense* \times *harknessii* it is apparent that more of the bivalent chromosomes are united at both ends than in hybrids between *G. hirsutum* or *G. contortum* and *G. armourianum*. The majority of pairs are more or less ring-shaped and several exhibit three chiasmata. It is also apparent that the unions between bivalent partners are comparatively stronger than those occurring in *G. hirsutum* \times *armourianum*.

HYBRIDS BETWEEN CULTIVATED AMERICAN AND WILD AUSTRALIAN SPECIES (N 26 \times N 13)

The meiotic divisions of *Gossypium barbadense* \times *sturtii* are very irregular. In the heterotypic division there is so much variation that in many pollen mother cells the type of conjugation could not be accurately determined. The following data for the first metaphase chromosome conjugation in 45 pollen mother cells, however, are believed to be accurate and characteristic of the majority of pollen mother cells.

Type of conjugation ¹	Number of pollen mother cells
0II+39I-----	25
1II+37I-----	8
2II+35I-----	5
3II+33I-----	5
4II+31I-----	2

An examination of the foregoing data shows that from 0 to 4 bivalent and from 31 to 39 univalent chromosomes are formed. Although the bivalents lie in the equatorial region, they do not form a definite plate. Their partners are united by a single chiasma, which often appears imperfect. In the majority of cases, the univalents are uniformly scattered over the achromatic figure. Figure 8 depicts a first metaphase having 2 bivalent and 35 univalent chromosomes.

The remaining stages of meiosis are similar to those found in *Gossypium hirsutum* \times *armourianum*. In *G. barbadense* \times *sturtii*, however, the irregularities are of greater magnitude. Also in the latter hybrid several somatic (39-chromosome) second-metaphase plates have been observed. At the completion of meiosis the sporads con

¹ The subscripts I and II are cytological symbols designating univalent and bivalent chromosomes.

tain from 2 to 14 spores, which vary in size from very small ones to those nearly twice the size of the tetrad spores of the *barbadense* parent.

HYBRIDS BETWEEN WILD AMERICAN AND WILD AUSTRALIAN SPECIES ($N\ 13 \times N\ 13$)

The types of conjugation at first metaphase in *Gossypium sturtii* \times *armourianum*, *G. sturtii* \times *harknessii*, and *G. davidsonii* \times *sturtii* are



FIGURE 8.—*Gossypium barbadense* \times *sturtii*. Profile view of first metaphase in a pollen mother cell, showing 2 bivalent and 35 univalent chromosomes. Four of the univalent chromosomes are in process of fragmentation.

given in table 3. Although chromosome pairing is variable in these hybrids, they exhibit a larger number of pairs than does *G. barbadense* \times *sturtii*, and pairing is more frequent. Table 3 indicates that from 0 to 6 bivalent and from 14 to 26 univalent chromosomes are formed in hybrids between the wild American species and *G. sturtii*. The arrangement of bivalent and univalent chromosomes on the achro-



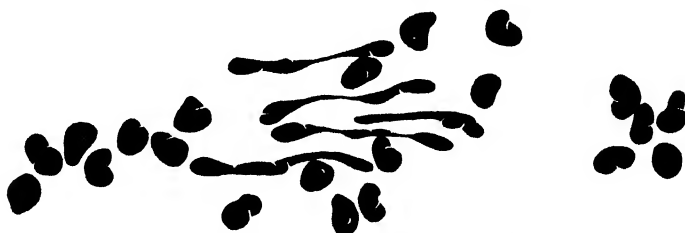
FIGURE 9.—*Gossypium sturtii* \times *armourianum*. Profile view of first metaphase in a pollen mother cell, showing 6 bivalent and 14 univalent chromosomes. Three of the univalent chromosomes are undergoing fragmentation.

matic figure is similar to that recorded in the case of *G. barbadense* \times *sturtii*. Also, like the latter hybrid, the bivalent partners are united at one end by a single chiasma, which is comparatively imperfect. Figure 9 depicts a first metaphase of *G. sturtii* \times *armourianum* having 6 bivalent and 14 univalent chromosomes; 3 of the latter are apparently fragmenting. Figure 10 shows a first metaphase of *G. davidsonii* \times *sturtii* having 26 chromosomes.

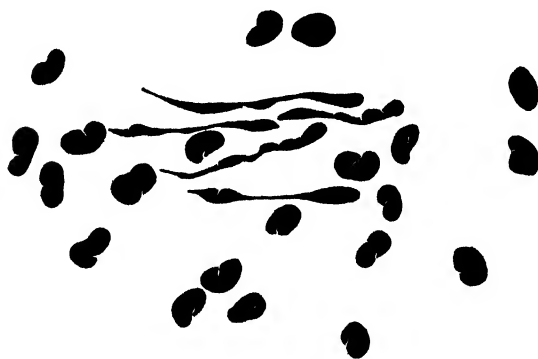
TABLE 3.—First-metaphase chromosome conjugation in pollen mother cells of hybrids between *Gossypium sturtii* and wild American species

Hybrid	Pollen mother cells with indicated type of conjugation						
	0 _{II} +26 _I	1 _{II} +24 _I	2 _{II} +22 _I	3 _{II} +20 _I	4 _{II} +18 _I	5 _{II} +16 _I	6 _{II} +14 _I
<i>G. sturtii</i> × <i>armourianum</i>	Number 26	Number 13	Number 4	Number 3	Number 1	Number 1	Number 2
<i>G. sturtii</i> × <i>harknessii</i>	11	4	1	1	1	1	1
<i>G. davidsonii</i> × <i>sturtii</i>	17	5	4	3	2	2	2

The irregularities occurring in the remaining stages of meiosis are similar to those exhibited by *Gossypium hirsutum* × *armourianum*,

FIGURE 10.—*Gossypium davidsonii* × *sturtii*. Profile view of first metaphase in a pollen mother cell, showing 26 univalent chromosomes, 5 of which are undergoing fragmentation.

but are somewhat smaller and considerably less pronounced in these hybrids than in *G. barbadense* × *sturtii*. Although the sporads of all three hybrids in this group are generally highly abnormal, those of *G. sturtii* × *armourianum* most nearly approach the tetrad.

FIGURE 11.—*Thurberia thespesioides* × *Gossypium sturtii*. Profile view of first metaphase in a pollen mother cell, showing 26 univalent chromosomes, 5 of which are fragmenting.

HYBRIDS BETWEEN THURBERIA THESPESIOIDES AND GOSSYPIMUM STURTII (N 13 × N 13)

During the first metaphase of *Thurberia thespesioides* × *Gossypium sturtii* there is no visible chromosome pairing. The 26 univalent chromosomes are scattered over the achromatic figure. Now and then several long slender univalents are found in the equatorial region. Although these

latter univalents occasionally divide, they usually exhibit a random distribution along with the remaining univalents. The homotypic divisions are quite regular, even though a few chromosomes often lag. The sporads are highly abnormal. Figure 11 depicts a characteristic first metaphase of *T. thespesioides* × *G. sturtii*.

A NATURAL HYBRID BETWEEN AN ASIATIC AND A CULTIVATED AMERICAN SPECIES
(N 13 × N 26)

The writer has made a careful cytological study of a natural hybrid between an Asiatic and a cultivated American species of *Gossypium* ($n_{13} \times n_{26}$). The particular Asiatic species to which one of the parents of this hybrid belonged is unknown. The American parent probably was *Gossypium hirsutum*. Longley (19) reported that this natural hybrid exhibited variable pairing, and he figured a metaphase with 14 bivalent and 11 univalent chromosomes. In the same hybrid Skovsted (24) reported the pairing in two pollen mother cells to be, respectively, 13 univalents, 8 bivalents, 1 tetravalent, and 1 hexavalent; and 14 univalents, 8 bivalents, 1 trivalent, and 1 hexavalent.

The writer's studies indicate that the meiotic behavior of this natural hybrid closely approaches that reported by Nakatomi (21) in similar hybrids and resembles the behavior of *Gossypium hirsutum* × *armourianum* reported above. Although during diakinesis there are generally 13 pairs and 13 single chromosomes, occasionally 1 to 3 quadrivalents are present. The majority of pairs are united at one end by 1 or 2 chiasmata. At least 4 pairs exhibit 3 chiasmata. Although several pairs display interstitial chiasmata, the majority have only subterminal or terminal ones. At the approach of the first metaphase the degree of terminalization of the chiasmata appears to vary. It is usually complete, but occasionally subterminal, and very rarely interstitial chiasmata occur. In several cases, the union of rod-shaped pairs is comparatively imperfect.

Following are the data for the first-metaphase chromosome conjugation in 62 pollen mother cells of this natural hybrid in F_2 .

Type of conjugation	Number of pollen mother cells
13 _{II} + 13 _I	38
12 _{II} + 15 _I	5
11 _{II} + 17 _I	3
10 _{II} + 19 _I	3
9 _{II} + 21 _I	1
1 _{IV} + 11 _{II} + 13 _I	7
2 _{IV} + 9 _{II} + 13 _I	2
3 _{IV} + 7 _{II} + 13 _I	1
4 _{IV} + 6 _{II} + 11 _I	2

An examination of the foregoing data reveals that out of 62 pollen mother cells 12 contained from 1 to 4 quadrivalent chromosomes and 38 had 13 bivalent and 13 univalent chromosomes. The remaining 12 pollen mother cells displayed a decrease in the amount of pairing. As in the case of *Gossypium hirsutum* × *armourianum*, where pairing is somewhat variable, when the maximum number of bivalents are present several of them exhibit comparatively poor or imperfect unions. Since the number of bivalents exhibiting imperfect unions decreases as the number of univalents increases, there can be little doubt that such unions are occasionally ineffectual. Figure 12 depicts a first metaphase having 2 quadrivalent, 9 bivalent, and 13 univalent chromosomes.

The remaining characteristics in meiosis resemble those displayed by *Gossypium hirsutum* × *armourianum*. However, in this natural hybrid, among the highly abnormal sporads, diads are fairly common.

DISCUSSION

INCOMPATIBILITY BETWEEN SPECIES OF *GOSSYPIMUM*

The degree of compatibility between different species is commonly supposed to correspond with their nearness of relationship, as indicated by their degree of morphological similarity. In *Gossypium* it is evident that such a supposition is not always true. Interspecific hybrids within the cultivated American group of cottons are easily obtained, while those between species within the Asiatic group are fairly difficult to obtain. Although the Asiatic and cultivated American species are not very dissimilar morphologically, hybrids between them are very difficult to obtain. On the other hand, *G. sturtii*, which is morphologically one of the most distinct species, readily hybridizes with species of all other groups of *Gossypium*.



FIGURE 12.—A natural hybrid between an Asiatic and a cultivated American cotton. Profile view of first metaphase in a slightly crushed pollen mother cell, showing 2 quadrivalent, 9 bivalent, and 13 univalent chromosomes. Two of the univalent chromosomes are in process of fragmentation.

The wild American cottons, although less dissimilar to the cultivated cottons than is *G. sturtii*, do not hybridize with them as freely as does *G. sturtii*.

NONVIABILITY OF THE GAMETES AND THE ZYGOTE IN INTERSPECIFIC HYBRIDS

In interspecific *Gossypium* hybrids the relationship of the parental species, as indicated by morphological characters, and the normality of the heterotypic division are usually correlated. In turn, the degree of fertility is correlated with the amount of chromosome pairing. It is logical to suppose that sterility in *Gossypium* hybrids is due to the production of nonviable gametes. In such hybrids as *G. sturtii* \times *harknessii*, *Thurberia thespesioides* \times *G. sturtii*, and *G. barbadense* \times *sturtii* the parental species are distantly related and the hybrids are completely sterile. In these hybrids there is little or no chromosome pairing during the heterotypic division. The chromosomes are irregularly distributed to daughter nuclei and some are

left in the plasma. On the other hand, the cultivated American species and the wild American species are presumably more closely related than the species involved in the hybrids with *G. sturtii*. In *G. barbadense* \times *harknessii* and *G. hirsutum* \times *armourianum* this supposition of relationship is borne out by distinct chromosome pairing. Although one would expect a greater production of viable gametes in the latter hybrids, the plants are completely sterile. The expectation is approached only in the formation of seedless capsules.

Interspecific hybrids within the cultivated American group of cottons exhibit complete chromosome pairing and are highly fertile. Likewise, the hybrid *Gossypium herbaceum* \times *arboreum* var. *neglectum*, between species of the Asiatic group, exhibits complete chromosome pairing and is highly fertile. However, the amount of chromosome pairing in the inter-Asiatic hybrid *G. arboreum* var. *sanguineum* \times *africanum* is slightly reduced and the fertility is correspondingly less.

Apparently, in crosses involving *Thurberia thespesioides*, a near relative if not actually a member of the genus *Gossypium*, there is a high degree of zygote mortality. Harland and Atteck (11) found that crosses between *G. stocksii* (a wild Asiatic species with $n=13$) and *Thurberia* were quite easily made, but that the hybrid seedlings were weak and died after 2 or 3 leaves were formed. The same authors found a similar condition in crosses between *G. davidsonii* and *Thurberia*. However, by using as pistillate parents 2 complex American hybrids, these authors obtained 8 healthy hybrids; and no mention is made of weak plants or of mortality. In the writer's experiments 10 percent of the cross-pollinations between *Thurberia* and *G. sturtii* produced good capsules. Approximately 50 percent of the hybrid seeds germinated and 66 percent of the seedlings grew to be healthy plants. Likewise, seedlings of hybrids between *G. hirsutum* and *Thurberia* are easily obtained, but they are extremely weak and die during early stages of development.

INHERITANCE IN INTERSPECIFIC HYBRIDS

In *Gossypium* there are relatively few reported cases of mono-hybrid segregation. Kearney (14) expresses the belief that this is partly due to the fact that most investigators have considered mainly characters of economic importance, such as size of the bolls and length and abundance of the lint, which are conditioned by several factors. Another reason, suggested by Harland, is that genetic analyses have dealt largely with interspecific rather than intraspecific hybrids. Harland (8, 9) found that several characters which exhibited alternative inheritance in intraspecific hybrids exhibited a "quantitative" type of inheritance in interspecific hybrids. He attributes the latter behavior to modifying factors, which have the effect of obscuring the segregation of characters.

Very little is known regarding the inheritance of the characters in the interspecific hybrids under discussion. Since back-crossed or selfed generations have not been grown, genetical analyses are wanting. However, several characters show a tendency to dominance in these hybrids between species. In color, the dark-red plant body in *Gossypium arboreum* var. *sanguineum* and *G. schottii* dominates the green plant body of other species. Likewise, the pinwheel appearance of the corolla spots in *G. sturtii* appears to dominate the type of corolla

spots characteristic of other species. The narrowly lobed leaf of *Thurberia* partially dominates the entire leaf of *G. sturtii*. To a lesser degree the lobed leaf of *G. hirsutum* dominates the entire leaf of *G. armourianum*. The presence of a petal spot in *G. sturtii*, *G. barbadense*, and *G. armourianum* tends to be dominant over absence of petal spot in other species. Similarly, colored pollen is partially dominant over uncolored pollen.

Interspecific *Gossypium* hybrids derived from species having the same number of chromosomes usually exhibit an intermediate expression of most of the contrasting characters of the parents. Such a condition is probably due to the lack of dominance or to an equal number of dominant factors being contributed by each parent. On the other hand, hybrids between species having, respectively, 13 and 26 as the haploid number of chromosomes resemble the parent having 26 haploid chromosomes. This is probably due to the transmission of a greater number of dominant factors along with the larger number of chromosomes. Except for the shape of the young parthenocarpic bolls in F_1 of *G. barbadense* \times *harknessii*, the *barbadense* parent appears to dominate *harknessii*. The decided resemblance of this hybrid to *G. barbadense* may be due to (1) the transmission from the *barbadense* parent of a greater number of dominant factors along with the larger number of chromosomes or (2) close genetic similarity of the *harknessii* chromosomes to 13 of the *barbadense* chromosomes (25). In the latter case, the *harknessii* chromosomes are sufficiently homologous to certain *barbadense* chromosomes to be combined with them without disturbing the *barbadense*-like nature of the hybrid.

PHYLOGENETIC CONCEPTIONS

Gates (7) has recently reviewed the literature bearing upon the origin of species in the genus *Gossypium*. He points out that both Davie and Skovsted have suggested that the species with 13 pairs of chromosomes are modified tetraploids. Gates also calls attention to the fact that the best evidence in support of this suggestion is found in the observations by Davie of secondary chromosome association in *G. herbaceum*. Neither Webber (27) nor Skovsted (23) have found secondary association within the Asiatic species. That there are no homologous chromosomes in the haploid sets of *Thurberia thespesioides* and *G. sturtii* is shown by the lack of chromosome pairing in hybrids between these two species. On the other hand, the slight amount of pairing in the hybrids between *G. sturtii* and the wild American species may indicate that certain chromosomes within the haploid set of the wild American species and of *G. sturtii* are homologous. In this case, however, it remains to be determined whether the pairing is within the wild American haploid set of chromosomes or between chromosomes of the parental species.

Skovsted (24) and Davie (3) have also suggested that the cultivated American cottons are allotetraploids. Skovsted concluded that their origin may be ascribed to the doubling of the chromosomes in a hybrid between an Asiatic and a wild American species. That the chromosomes of the wild American and of the Asiatic species are homologous to 13 of the chromosomes in the cultivated American cottons is indicated by the occurrence of 13 pairs of chromosomes in the hybrids *Gossypium hirsutum* \times *armourianum*, *G. barbadense* \times *harknessii*

and the natural hybrid between an Asiatic and a cultivated American species. The fact that *G. barbadense* \times *sturtii* rarely exhibits chromosome pairing and never shows a high degree of conjugation indicates that the pairing which occurs in the F_1 of the hybrids *G. hirsutum* \times *armourianum*, *G. barbadense* \times *harknessii*, and of the natural hybrid between an Asiatic and a cultivated American species is between the chromosomes of the species involved in the hybrids rather than between chromosomes within the cultivated American species. Before Skovsted's suggestion can be accepted, it remains to be proved that the chromosomes of the Asiatic and the wild American species are nonhomologous.

Davie believes that the occurrence of several well-isolated wild American species characterized by 13 pairs of chromosomes indicates that the American species with 26 pairs probably arose through chromosome doubling in crosses between different wild American species. It was shown in the preceding paragraph that the chromosomes of *Gossypium harknessii* and *G. armourianum* are homologous to 13 of those of the cultivated American species. That this homology is between the same set of chromosomes in either *G. hirsutum* or *G. barbadense* is indicated by the fact that complete chromosome pairing occurs in F_1 of both *G. hirsutum* \times *barbadense* and *G. harknessii* \times *armourianum*.

Among the wild American species that may be involved in the origin of cultivated American cottons is *Thurberia thespesioides*. The writer finds that the somatic chromosomes of this species are slightly larger than those of the other wild American species. Skovsted (24) reports that the chromosomes of the cultivated American cottons may be equally divided into a larger and a smaller group. The differences of chromosome dimensions in cotton are very slight, however, and the writer's observations indicate a gradation in size of the chromosomes of the cultivated American species rather than a sharp segregation of large and small ones.

In the cultivated American species Webber (27) found that during meiosis quadrivalent chromosomes are occasionally formed. If the assumption that these species were derived from a cross between two modified tetraploids is correct, then such quadrivalent formation is easily explained upon the basis of chromosome homologies known to exist in similar modified octoploids. Such an explanation would also account for the occasional formation of quadrivalents in (1) hybrids between the species of the cultivated American group of cottons, (2) hybrids between the latter species and the wild American species, and (3) the natural hybrid between an Asiatic and a cultivated American species.

In the natural hybrid between an Asiatic and a cultivated American species, the composition of the pollen mother cells exhibiting quadrivalent chromosomes was found to be $1_{IV}+11_{II}+13_I$, $2_{IV}+9_{II}+13_I$, $3_{IV}+7_{II}+13_I$, or $4_{IV}+6_{II}+11_I$. Except in the last class, the quadrivalent group is followed by a decrease of 2 in the bivalent group, and in these three classes the number of univalents remains constant. Hence, it seems logical to assume that the quadrivalents are composed of chromosomes that generally form bivalents. If such an assumption is correct, then one of the bivalent chromosomes in the exceptional class just mentioned must be composed of chromosomes that generally form univalents.

SUMMARY AND CONCLUSIONS

In *Gossypium*, cross-pollinations made in the morning are more successful than those made in the afternoon. Those made in the late summer are more successful than those made in early summer. By removing the entire corolla and androecium during emasculation, rather than the anthers only, the percentage of successful cross-pollinations is greatly increased.

The correlation of the degree of compatibility between different species with their morphological similarity is often very limited. It is often less difficult to obtain hybrids between species of different taxonomic groups than between species of the same taxonomic group.

Hybrids between and within the following five morphologically distinct groups of *Gossypium* are described: (1) Cultivated American species ($n=26$); (2) wild American species ($n=13$); (3) cultivated Asiatic species ($n=13$); (4) a wild Australian species, *G. sturtii* ($n=13$); and (5) *Thurberia thespesioides* ($n=13$) a wild American plant, possibly congeneric with *Gossypium*.

Usually, in interspecific *Gossypium* hybrids the morphological similarity of the parental species and the normality of the heterotypic divisions are correlated. Also, the degree of fertility is correlated with the amount of chromosome pairing.

Certain characters show a tendency to dominance in interspecific hybrids of *Gossypium*. Other characters are expressed in a more or less intermediate degree.

Interspecific hybrids within the same group exhibit normal meiotic behavior. Hybrids between species having 26 pairs of chromosomes, like their parents, occasionally form quadrivalent chromosomes. Although hybrids between cultivated American and wild American species generally form 13 bivalent and 13 univalent chromosomes, they also occasionally form quadrivalent chromosomes. The latter behavior is also characteristic of a natural hybrid between an Asiatic and a cultivated American cotton. *Gossypium barbadense* \times *sturtii* usually exhibits no chromosome pairing, but occasionally as many as 4 bivalent chromosomes have been observed. Hybrids between wild American species and *G. sturtii* likewise exhibit variable pairing. In the latter case, however, pairing is more frequent. Hybrids between *Thurberia thespesioides* and *G. sturtii* exhibit no chromosome pairing.

The occurrence of limited chromosome pairing in hybrids between wild American species and *Gossypium sturtii* and the formation of quadrivalents in hybrids between cultivated American and wild American species, and in a natural hybrid between an Asiatic and a cultivated American cotton, seem to support the hypothesis that the species having 13 pairs of chromosomes are modified tetraploids. If such is the case, then the lack of pairing in *Thurberia thespesioides* \times *G. sturtii* must indicate that the chromosomes within the haploid sets of these two species have differentiated genetically to such an extent that pairing within either haploid set is impossible.

The formation of 13 bivalent chromosomes in hybrids between cultivated American and wild American species and in a natural hybrid between an Asiatic and a cultivated American cotton and the very limited pairing in *Gossypium barbadense* \times *sturtii* support the hypothesis that the species having 26 pairs are allotetraploids. Such

an origin possibly involved species or close allies of some two of the following groups: Wild American species of *Gossypium*, *Thurberia hespesioides*, and Asiatic species of *Gossypium*.

LITERATURE CITED

- (1) BARANOV, P.
1930. COTTON PLANT: CYTOLOGY. PLANT BREEDING. Bull. Sci. Research Cotton Inst. Tashkent 5: 7-17. Abstract in Plant Breeding Abs. 2: 197-198, 1932. [Original not seen. Abstract in Jour. Textile Inst. 23: A475. 1932.]
- (2) COOK, O. F., and HUBBARD, J. W.
1926. NEW SPECIES OF COTTON PLANTS FROM SONORA AND SINALOA, MEXICO. Jour. Wash. Acad. Sci. 16: 333-339.
- (3) DAVIE, J. H.
1933. CYTOLOGICAL STUDIES IN THE MALVACEAE AND CERTAIN RELATED FAMILIES. Jour. Genetics 28: [33]-67, illus.
- (4) DENHAM, H. J.
1924. THE CYTOLOGY OF THE COTTON PLANT. II. CHROMOSOME NUMBER OF OLD AND NEW WORLD COTTONS. Ann. Bot. [London] 38: [433]-438, illus.
- (5) DESAI, B. B.
1927. A CROSS BETWEEN INDIAN AND AMERICAN COTTONS. Agr. Jour. India 22: 351-353.
- (6) DOAK, C. C.
1934. A NEW TECHNIQUE IN COTTON HYBRIDIZING. SUGGESTED CHANGES IN EXISTING METHODS OF EMASCULATING AND BAGGING COTTON FLOWERS. Jour. Heredity 25: 201-204, illus.
- (7) GATES, R. R.
1934. THE CYTOLOGICAL STUDY OF COTTON AND ITS RELATIVES. Empire Cotton Growing Rev. 11: 194-201, illus.
- (8) HARLAND, S. C.
1929. THE GENETICS OF COTTON. PART II. THE INHERITANCE OF POLLEN COLOUR IN NEW WORLD COTTONS. Jour. Genetics 20: [387]-399, illus.
- (9) ———
1930. COTTON NOTES. RECENT WORK ON THE GENETICS OF COTTON. Trop. Agr. [Trinidad] 7 (1): 16-18.
- (10) ———
1932. THE GENETICS OF GOSSYPIMUM. Bibliographia Genetica 9: [107]-182.
- (11) ——— and ATTECK, O. S.
1931. INTERGENERIC HYBRIDS BETWEEN GOSSYPIMUM AND THURBERIA. Amer. Nat. 65: 380-382.
- (12) KEARNEY, T. H.
1922. THE UNIFORMITY OF PIMA COTTON. U. S. Dept. Agr. Circ. 247, 6 pp.
- (13) ———
1923. SELF-FERTILIZATION AND CROSS-FERTILIZATION IN PIMA COTTON. U. S. Dept. Agr. Bull. 1134, 68 pp., illus.
- (14) ———
1930. GENETICS OF COTTON. A SURVEY OF OUR PRESENT KNOWLEDGE. Jour. Heredity 21: 325-336, 375-384, 409-415, illus.
- (15) ———
1933. A NEW GOSSYPIMUM OF LOWER CALIFORNIA. Jour. Wash. Acad. Sci. 23: 558-560.
- (16) ———
1934. AMERICAN WILD COTTONS WITH THIRTEEN CHROMOSOMES. Jour. Heredity 25: 305-312, illus.
- (17) ——— and PORTER, D. D.
1926. BAGGING COTTON FLOWERS TO PREVENT ACCIDENTAL CROSS-POLLINATION. Jour. Heredity 17: 273-279, illus.
- (18) LAWRENCE, W. J. C.
1931. THE SECONDARY ASSOCIATION OF CHROMOSOMES. Cytologia (Tokyo) 2: 352-384, illus.

- (19) LONGLEY, A. E.
1933. CHROMOSOMES IN *GOSSYPIMUM* AND RELATED GENERA. *Jour. Agr. Research* 46: 217-227, illus.
- (20) MATSUURA, H.
1929. A BIBLIOGRAPHICAL MONOGRAPH ON PLANT GENETICS (GENIC ANALYSIS) 1900-1923. 499 pp. Tokyo. (Tokyo Imp. Univ., Bot. Inst. Contrib. Cytology and Genetics, no. 82).
- (21) NAKATOMI, S.
1931. HYBRIDIZATION BETWEEN OLD WORLD AND NEW WORLD COTTON SPECIES AND THE CHROMOSOME BEHAVIOR OF THE POLLEN MOTHER CELLS IN THE F_1 HYBRID. *Japan. Jour. Bot.* 5: [371]-383, illus.
- (22) OAKLEY, R. A.
1915. DISTRIBUTION OF COTTON SEED 1915. U. S. Dept. Agr., Bur. Plant Indus. Doc. 1163, 15 pp.
- (23) SKOVSTED, A.
1933. CYTOLOGICAL STUDIES IN COTTON. I. THE MITOSIS AND MEIOSIS IN DIPLOID AND TRIPLOID ASIATIC COTTON. *Ann. Bot. [London]* 47: [227]-251, illus.
- (24) ———
1934. CYTOLOGICAL STUDIES IN COTTON. II. TWO INTERSPECIFIC HYBRIDS BETWEEN ASIATIC AND NEW WORLD COTTONS. *Jour. Genetics* 28: [407]-424, illus.
- (25) WATKINS, A. C.
1925. GENETIC AND CYTOLOGICAL STUDIES IN WHEAT. II. *Jour. Genetics* 15: [323]-366, illus.
- (26) WATT, G.
1907. THE WILD AND CULTIVATED COTTON PLANTS OF THE WORLD; A REVISION OF THE GENUS *GOSSYPIMUM*, FRAMED PRIMARILY WITH THE OBJECT OF AIDING PLANTERS AND INVESTIGATORS WHO MAY CONTEMPLATE THE SYSTEMATIC IMPROVEMENT OF THE COTTON STAPLE. 406 pp., illus. London, New York [etc.].
- (27) WEBBER, J. M.
1934. CHROMOSOME NUMBER AND MEIOTIC BEHAVIOR IN *GOSSYPIMUM*. *Jour. Agr. Research* 49: 223-237, illus.
- (28) ———
1934. CYTOGENETIC NOTES ON COTTON AND COTTON RELATIVES. *Science* (n. s.) 80: 268-269.
- (29) ZAITZEV, G. S.
1924. A HYBRID BETWEEN ASIATIC AND AMERICAN COTTON PLANTS, *GOSSYPIMUM HERBACEUM* L. AND *GOSSYPIMUM HIRSUTUM* L. *Trudy Prikl. Bot. i. Selekt. (Bull. Appl. Bot. and Plant Breeding)* 13 (2): 117-134. [In Russian. English summary, pp. 132-134.]

OCCURRENCE OF SELENIUM IN NATURAL PHOSPHATES, SUPERPHOSPHATES, AND PHOSPHORIC ACID¹

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INTRODUCTION

The occurrence, origin, and distribution of selenium in soils, its absorption by plants, and the toxicity of the plants to animals have been discussed in several recent papers (3, 7, 8, 9, 13, 18).³

With regard to the limits in the quantities of selenium that may be present in soil and vegetation without injury to animals, Byers (3, p. 44) states:

In general it would appear that any soil containing upwards of 0.5 p. p. m. of selenium, and any vegetation containing 5 p. p. m. is potentially dangerous.

More than a decade ago Stoklasa (22) pointed out that the selenium content of soil may be greatly increased through heavy fertilization with superphosphate and ammonium sulphate. That the quantities of selenium occurring in fertilizers and soil amendments is sufficient to alter the amount the plant would otherwise absorb from the soil appears possible, when due consideration is given to the fact that these agents are applied to the layers of soil most readily accessible to the roots of the plant during its growing period, and often, particularly in the case of fertilizers, in relatively large quantities very near the root system. For example, let it be supposed that a fertilizer containing 50 p. p. m. of selenium is applied to the soil at the rate of 1,000 pounds per acre within 3 inches of the plant rows which are 3 feet apart. Then, assuming the customary figure, 2,000,000 pounds of topsoil to a depth of 6 inches per acre, the increase in the selenium concentration of the entire soil layer would be 0.025 p. p. m., and the increase in the concentration in a 6-inch layer of topsoil extending 3 inches on either side of the row, if all the selenium were retained therein, would be about 0.15 p. p. m. Under certain conditions the latter figure may represent a significant increase in the selenium content of the soil.

Results are given in this paper for selenium in 96 representative samples of phosphate rock and 3 samples of apatite from various deposits of the world, 8 typical samples of domestic superphosphate, and 4 samples of crude phosphoric acid manufactured by the sulphuric acid process. The results for selenium in natural phosphates reported herein represent an extension of the studies of this Bureau on the composition of phosphate rock (11, 16).

PROCEDURE AND REAGENTS USED

The procedure used in the determination of selenium was substantially the same as that developed by Robinson, Dudley, Williams, and Byers (19) for selenium in soil and in sulphide-bearing rocks

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³ Reference is made by number (italic) to Literature Cited, p. 1082.

and minerals, according to which the selenium is separated and concentrated by distillation with hydrobromic acid in the presence of bromine and is determined in the distillate by gravimetric methods, or by a colorimetric procedure when the absolute amount of selenium is less than 0.5 mg. Three distillation procedures are described for inorganic materials: (1) Direct distillation of the sample, applicable to soil and nonpyritiferous rocks and minerals; (2) distillation after the sample has been pretreated with nitric acid to destroy sulphides, and finally with concentrated sulphuric acid to eliminate the nitrate radical which otherwise would decompose the hydrobromic acid in the subsequent distillation, applicable to materials containing pyrite and other sulphides; and (3) integrated distillation, a method of concentration in which the distillate from one portion of the sample is added in the distillation of a fresh portion and so on, which is especially adapted to the determination of traces of selenium.

Phosphate rock presents some difficulties in the application of these methods. As a result of the presence of considerable quantities of fluorine and silica, relatively large quantities of gelatinous silica are rapidly deposited in the condenser during the early stages of distillation, and when more than about 10 g of the samples are used, the condenser often becomes clogged. Furthermore, the amount of gelatinous silica that separates in the distillate when large samples are used is sufficient seriously to retard subsequent filtration. These difficulties are intensified in the integrated distillation. For a calcium-rich material like phosphate rock, sulphuric acid is not satisfactory for the expulsion of the nitrate radical following the preliminary treatment of pyritiferous samples, because the relatively insoluble calcium sulphate causes serious bumping during subsequent distillation. Phosphoric acid is recommended for this purpose.

Selenium can be removed from dry soil by distillation with hydrobromic acid with a specific gravity as low as 1.2.⁴ Accordingly, acid sufficiently concentrated for use in the distillation can be readily recovered by redistilling the filtrates and solutions from the estimation of the selenium. The specific gravity of the recovered acid used by the writers ranged between 1.35 and 1.40. When 10 g of 100-mesh pyrite-free rock was distilled with 100 ml of acid of this strength, all the selenium passed into the first 50 ml of the distillate. In samples that carried only small amounts of acid-insoluble sulphides, selenium was detected in the second 50 ml of the distillate but not thereafter. In view of these findings the volume of distillate collected was 75 to 150 ml, depending on the character of the sample. It should be mentioned that the difficulties with the separation of gelatinous silica are less marked when the more dilute acid is used.

Bromine often contains impurities capable of imparting to the distillate a color which, since it persists after the solution is reduced with sulphur dioxide, interferes in the colorimetric comparison. The interfering substance can be eliminated by dissolving the bromine in hydrobromic acid, filtering off the flocculent precipitate,⁵ and distilling the filtered solution.

⁴ BYERS, H. G. Private communication.

⁵ The nearly white, waxy substance (0.232 g of air-dried material) obtained by adding 30 ml of bromine to 800 ml of water-white hydrobromic acid (48 percent), allowing the mixture to stand an hour, filtering, and washing the precipitate with cold water, was soluble in alcohol and ether. Somewhat more than one-half of the substance melted at 136° to 140° C.; the remainder did not melt at 160° C. When it was subjected to steam distillation about two-thirds of the wax passed over with the steam.

In order to compare the performance of the three distillation methods mentioned, these procedures were used to determine the selenium in several typical phosphate rocks. The results (table 1) indicate that, even though the rock contains relatively large quantities of organic matter, pretreatment of the sample is not necessary, unless acid-insoluble sulphides are present and then only when the highest accuracy is desired. Unless it is stated otherwise, the results for selenium reported in this paper were obtained by distilling the samples without pretreatment.

TABLE 1.—Selenium in typical phosphate rocks as determined by three different distillation procedures

Sample no.	Type or source of phosphate	FeS ₂	Organic carbon	Selenium determined by—		
				Distilling 10 g of sample		Integrated distillation of untreated sample ¹
				Un-treated	Pretreated	
		Percent	Percent	P. p. m.	P. p. m.	P. p. m.
973	Idaho.....	0.00	2.34	28	28	(²)
948	Wyoming.....	.97	3.47	48	48	(²)
449	Tennessee blue rock.....	2.39	(²)	8	1.0	(²)
930	do.....	3.90	.20	2.0	3.0	(²)
1139	South Carolina land rock.....	.36	(²)	14	16	(²)
912	Florida land pebble.....	.00	.38	8	(²)	0.8
908	Tennessee brown rock.....	.00	.11	8	(²)	<.1
916	Tennessee phosphatic limestone.....	.73	(²)	8	(²)	<.1
917	do.....	1.89	(²)	8	(²)	.2

¹ 100 g of the sample was distilled in 10-g portions.

² Not determined.

Under the conditions prescribed (19) for the colorimetric estimation, the threshold sensitivity of the method is about 0.008 mg of selenium. For 10 g of sample this figure corresponds to 0.8 p. p. m., and accordingly the results are reported as ≤ 0.8 p. p. m. in those cases where the coloration was barely perceptible and as < 0.8 where no color was noted. When 100 g of the sample are used (integrated distillation) the detectable concentration of selenium is thereby reduced to approximately 0.1 p. p. m.

SELENIUM IN NATURAL PHOSPHATES

Results for selenium in domestic and foreign phosphates are given in tables 2 and 3, respectively. All the samples from Florida, Tennessee (except Tennessee blue rock), Kentucky, Arkansas, Oklahoma, and Australia, the light-colored phosphates from the western part of the United States, and the apatites from Virginia and Canada contained 1 p. p. m. or less of selenium. The results for the dark-colored phosphates of the western part of the United States and Canada, the majority of the Tennessee blue-rock and South Carolina samples, and the African and Palestinian phosphates ranged between 1 and 55 p. p. m. A few of the samples from European and insular deposits contained as much as 1 to 2 p. p. m. of selenium.

TABLE 2.—Selenium content of domestic phosphates

FLORIDA PHOSPHATES

Sample no.	Type of phosphate	Location of deposit	P ₂ O ₅	Se
			Percent	P. p. m.
910	Land pebble	Mulberry	31.09	< 0.8
947	do	Brewster	31.28	< 0.8
790	do	Not known	31.40	< 0.8
912	do	Mulberry	35.37	1.8
771	Hard rock	Not known	31.25	< 0.8
589	do	Floral City	34.68	< 0.8
932	do	Dunnellon	35.99	< 0.8
1091	Soft	Bartow	25.47	< 0.8
728	do	Juliette	31.80	< 0.8
915	Waste pond	Dunnellon	23.63	< 0.8

SOUTH CAROLINA PHOSPHATES

495	Not known	Not known	16.07	< 0.8
1139	Land rock	Bulow mines, Johns Island	26.92	16.0
1138	do	Lamb's mine, near Charleston	27.85	8.5
650	do	Not known	28.86	5.0

TENNESSEE PHOSPHATES

56	Brown rock	Not known	31.28	< 0.8
906	do	Wales	34.39	< 0.8
908	do	Mountpleasant	34.44	< 1.1
772	Blue rock	Glover	30.45	< 3.0
930	do	Gordonsburg	30.97	< 3.0
448	do	Glover	32.03	< 2.5
449	do	Gordonsburg	32.03	< 1.0
1049	Kidney phosphate	Boma	31.22	< 0.8
1043	White rock	Toms creek	30.20	< 0.8
1051	do	Godwin	35.80	< 0.8
916	Phosphatic limestone	Mountpleasant	11.22	< 1.1
917	do	Gordonsburg	11.68	< 2

WESTERN PHOSPHATES

550	Light colored	Idaho, Paris	32.21	< 0.8
1412	do	do	35.39	1.0
1011	do	Montana, Harrison	27.63	< 0.8
1018	do	do	29.11	< 0.8
1019	do	do	31.47	< 0.8
1017	do	do	34.92	< 0.8
1012	do	do	36.07	< 0.8
1262	do	do	36.38	< 0.8
1010	do	do	37.47	1.0
1407	do	do	37.93	< 0.8
1411	Dark colored	Idaho, Georgetown	30.29	50
489	do	do	31.97	6.0
973	do	Idaho, Conda	31.97	28
1253	do	do	32.13	8.0
454	do	do	32.24	40
1408	do	do	32.26	23
494	do	Idaho, Georgetown	34.96	8.0
1280	do	Montana, Maxville	24.95	6.0
1410	do	Utah, Devils Slide	11.90	38
1409	do	Utah, Logan	31.50	10
467	do	Wyoming, Cokeville	26.60	35
469	do	do	29.75	50
468	do	do	29.79	55
948	do	do	30.19	48

OTHER PHOSPHATES

1267		Arkansas, Independence County	31.98	< 0.8
1235	Brown rock	Kentucky, Wallace	21.19	< 0.8
1242		Oklahoma, Cotton County	24.31	< 0.8
1295	Apatite	Virginia, Amherst County	39.58	< 0.8

¹ Result obtained by integrated distillation.

² Result obtained by distillation of pretreated sample.

³ National Bureau of Standards sample no. 56.

TABLE 3.—Selenium content of foreign phosphates

AFRICAN PHOSPHATES

Sample no.	Location of deposit	P ₂ O ₅	Se	Sample no.	Location of deposit	P ₂ O ₅	Se
		Per cent	P. p. m.			Per cent	P. p. m.
560	Algeria, Dyr.....	23.39	18.0	453	Morocco.....	33.47	6.0
551	Algeria, Tebessa.....	26.10	2.5	563	do.....	34.30	1.5
558	Algeria, Rebiba.....	26.84	3.0	1162	do.....	35.11	1.0
562	Algeria, M'Zaita.....	28.59	7.5	552	Tunis, Gafsa.....	26.91	18.0
557	Algeria, Tooqueville.....	29.38	18.0	556	Tunis, Kalaa-Djerda.....	27.55	30.0
559	Algeria, Bordj-Redir.....	32.34	55.0	561	Tunis, M'Dilla.....	28.66	8.5
555	Egypt, Kosseir.....	30.60	1.0	553	Tunis, Gafsa.....	29.13	1.0

EUROPEAN AND ASIAN PHOSPHATES

1226	Belgium, Liège.....	18.13	<0.8	1263	U. S. S. R., Saratov.....	18.40	<0.8
1155	Estonia, Tallinn.....	25.68	<.8	1260	U. S. S. R., Egoriev.....	19.50	1.5
1228	France, Somme.....	22.02	1.5	1264	do.....	22.37	1.0
1240	do.....	24.66	<.8	1262	U. S. S. R., Vyatka.....	27.88	<.8
1241	France, Pyrenees Mountains.....	26.87	2.0	1260	U. S. S. R., Kola Peninsula.....	39.08	<.8
1239	France, Quercy.....	34.74	1.0	1258	Palestine, Neby Musa.....	8.14	1.5
1151	Portugal, Marvão.....	27.14	<.8	1255	do.....	16.60	1.0
1152	U. S. S. R., Volga River region.....	13.40	<.8	1256	do.....	17.50	1.5
1265	U. S. S. R., Aktyubinsk, Siberia.....	17.42	<.8	1257	do.....	20.40	15.0

INSULAR PHOSPHATES

1223	Angaur Island.....	40.00	<0.8	943	Curaçao Island.....	40.66	1.0
452	Christmas Island.....	39.46	<.8	1159	Makatea Island.....	38.22	<.8
904	Grand Connetable Island.....	54.51	2.0	450	Nauru Island.....	38.92	<.8
985	Curaçao Island.....	38.59	1.5	451	Ocean Island.....	40.32	<.8

OTHER PHOSPHATES

1157	South Australia, Kapunda.....	30.18	<0.8	2582	Canada, British Columbia.....	24.11	3.0
1158	South Australia, Port Clinton.....	33.53	<.8	1905	Canada, Quebec Province.....	40.30	.8

¹ Apatite.² A dark-colored phosphate from Crow's Nest Pass.

SELENIUM-BEARING CONSTITUENTS

In general, selenium is found in nature associated with sulphur, particularly the sulphides (4, 5, 17) and in a number of rare selenide minerals analagous to the sulphides (17, pp. 693-695). Byers (3) concluded that the selenium in soils came from sulphide minerals in the soil parent materials. Since selenium under certain conditions is absorbed by growing plants and is associated with the protein (18), organic matter originating from vegetation may be regarded as a potential source of selenium in sedimentary rocks. If the organic matter represents the remains of plant life that grew on seleniferous soil under climatic conditions favorable to selenium absorption, it may easily be the principal carrier of selenium.

In view of the foregoing some relationship may be expected to exist between the selenium content of phosphate rock and the quantities of sulphide sulphur, organic carbon, and nitrogen present. According to the comparative data (table 4), larger amounts of selenium occur in pyritiferous than in pyrite-free samples from the same region, and as a rule rocks containing the greater amounts of organic matter also carry the larger quantities of selenium. The latter relationship is best shown by the Permian phosphates of the western part of the

United States, and at least in the case of these phosphates it may be regarded as evidence that the organic matter is seleniferous. The nitrogen figures show somewhat less correlation with the selenium results than do the figures for organic carbon. The sample of Tennessee kidney phosphate is a notable exception to these general relationships.

TABLE 4.—Comparative results for selenium, organic carbon, nitrogen, and pyritic sulphur in phosphate rock

Source or type of phosphate	Organic carbon	Se	N	FeS ₂
	Percent	P. p. m.	P. p. m.	Percent
Western part of the United States:				
Dark-colored pyritiferous rock.....	13.90	130.0	1,100	1.92
Dark-colored pyrite-free rock.....	12.60	113.0	1,000	.0
Light-colored rock.....	1.16	<.9	80	.0
Tennessee kidney phosphate.....	1.46	11.8	2,600	1.5
Tunis, Gafsa.....	.86	18.0	470	.0
South Carolina.....	.51	8.5	510	.3
Florida.....	.31	<.8	170	.0
Grand Connetable Island.....	.28	2.0	490	.0
Tennessee blue rock.....	.28	11.4	280	3.0
Curaçao Island.....	.17	1.5	130	.0
Morocco.....	.17	1.0	180	.0
Tennessee brown rock and Tennessee white rock.....	.16	<.8	160	(³)

¹ Average of results for 3 or more samples.

² Average of results for 2 samples.

³ The 1 pyritiferous brown rock, National Bureau of Standards standard sample no. 56, contained sulphide equivalent to 0.89 percent of FeS₂.

Results for organic carbon and selenium in mechanical separates of ground phosphate rock are given in table 5. The correlation between the results for selenium and organic carbon is probably as close as could be expected, particularly in the sample of Wyoming phosphate. In the Idaho sample the results indicate considerable loss of selenium occasioned by suspension of the material in water incident to mechanical separation into fractions.

TABLE 5.—Distribution of selenium among mechanical separates of ground phosphate rock

Mechanical fraction ¹	Idaho phosphate no. 973			Wyoming phosphate no. 948			
	Fraction of original material	Organic carbon	Se	Fraction of original material	Organic carbon	Se	FeS ₂
	Percent	Percent	P. p. m.	Percent	Percent	P. p. m.	Percent
"Sand".....	26.0	2.22	33	49.8	2.96	36	0.62
"Silt".....	45.5	2.08	20	43.6	4.25	56	1.17
"Clay".....	27.9	3.08	18	6.6	8.29	64	.42
Solution loss.....	.6	-----	.5	.0	-----	.2	-----
Original material.....	100.0	2.34	28	100.0	3.47	48	.97

¹ The mechanical fractions of these phosphates were prepared by Alexander and Jacob (1) from material ground to pass a 100-mesh sieve.

² Calculated.

PRIMARY AND SECONDARY DEPOSITS

Following Blackwelder's division (2) of the world's phosphate deposits into six genetic varieties, comprised in two broad groups (primary and secondary deposits), Mansfield (15, p. 362) has partially

classified a number of the deposits. Accordingly, as far as possible the results for selenium in phosphate are summarized under the two general groups in table 6.

TABLE 6.—Selenium content of phosphate rocks from primary and secondary deposits

Type of deposits and location	Samples analyzed	Selenium	
		Range	Average
Primary:	Number	P. p. m.	P. p. m.
Western part of the United States and Canada.....	25	<0.8-55	<16.7
Algeria, Tunis, and Egypt.....	11	1.0-55	14.3
Tennessee (blue rock) and Arkansas.....	5	<.8-3.0	<1.6
Average.....			<10.9
Secondary:			
South Carolina.....	4	<.8-16	<7.6
France.....	4	<.8-2.0	<1.3
Islands.....	8	<.8-2.0	<1.1
Florida.....	10	<.8	<.8
Tennessee and Kentucky (brown rock and white rock).....	6	<.8	<.6
South Australia.....	2	<.8	<.8
Average.....			<2.0

Byers' conclusion (3) that selenium is leached from soil by percolating waters, which is supported by the presence of considerable amounts of this element in drainage water (table 8), and the fact that a considerable part of the selenium in one phosphate rock (table 5) was removed when the material was treated with water incident to mechanical analysis, afford good reasons to expect primary deposits, having been protected from the action of water, to carry larger quantities of selenium than secondary deposits. Considering the average figures for the groups (table 6), the primary deposits show about five times as much selenium as the secondary deposits, and the expectation is, in general, thus confirmed. It should be pointed out, however, that this criterion fails when it is applied to certain individual deposits. For example, South Carolina phosphate, a secondary deposit, carries about fivefold as much selenium as does Tennessee blue rock, a primary deposit. In this connection special interest attaches to the experimental finding (9) that the absorption and retention of added selenium is much more pronounced in some soils than in others, and to the suggestion that this difference in the behavior of the selenium might be due to the difference in the composition of the soil colloids.

GEOLOGIC AGE AND SELENIUM CONTENT

In table 7 the results for selenium in natural phosphates are grouped, as far as possible, according to the geologic age of the deposits (10). Of the deposits for which the average result is greater than 1 p. p. m., the Permian phosphates show the largest amount of selenium and the post-Tertiary the least. In the descending order of selenium content, the Miocene, Cretaceous (or Jurassic), Eocene, and Devonian phosphates are between these extremes.

TABLE 7.—*Geologic age as correlated with selenium content of natural phosphates*

Period or epoch	Source or type of phosphate	Samples analyzed	Selenium	
			Range	Average
		Number	P. p. m.	P. p. m.
Post-Tertiary.....	Island phosphates.....	8	<0.8-2.0	<1.1
Tertiary.....	Florida, South Carolina, Morocco, Australia, and Russia.....	20	<.8-16	<2.4
Pliocene.....	Florida land pebble.....	4	≡.8	<.8
Miocene.....	South Carolina land rock.....	3	5.0-16	9.8
Oligocene.....	Florida hard rock and soft phosphate.....	6	≡.8	<.8
Eocene.....	Morocco.....	3	1.0-5.0	2.5
Cretaceous (or Jurassic).....	France, Russia, Algeria, Tunis, and Egypt.....	19	<.8-55	<8.8
Carboniferous.....	Western United States and Canada.....	25	<.8-55	<16.7
Permian.....	Dark-colored phosphates ¹	15	3.0-55	27
Do.....	Light-colored phosphates ¹	10	<.8-1.0	<.8
Mississippian.....	Tennessee kidney phosphate.....	1		≡.8
Devonian.....	Tennessee blue rock.....	4	≡.8-31	≡.8
Ordovician.....	Tennessee and Kentucky brown rock ²	8	<.8	<.8
Pre-Cambrian.....	Apatite from Virginia and Canada.....	2	<.8	<.8

¹ From Canada; Georgetown, Idaho; Maxville, Mont.; Cokeville, Wyo.; and British Columbia.

² From Paris, Idaho; and Garrison, Mont.

³ Also 2 samples of Tennessee phosphatic limestone, 1 sample of Arkansas phosphate, and 1 sample of Estonian phosphate.

In view of previous discussion, it is hardly necessary to remark that other factors may play a greater part than geologic age in limiting the amounts of selenium that occur in any given deposit. As to the geologic age, a comparison of primary deposits is most logical. Accordingly, the geologic periods during which the primary phosphate deposits were laid down are, in the increasing order of average selenium content of the phosphates, Devonian, Cretaceous, and Permian.

PHOSPHATES COMPARED WITH OTHER ROCKS, MINERALS, SOIL, AND WATER

The available results for selenium in rocks, minerals, soils, and waters of the United States are summarized in table 8. The analyses of all the materials other than phosphates were recently published by Byers (3) and Williams and Byers (25). Since the data are by far the most extensive for certain localities west of the Mississippi River, mainly parts of Colorado, Montana, Nebraska, South Dakota, and Wyoming, the results have been classified under two geographic divisions of the country, the Mississippi River being used as the dividing line. The results of Goldschmidt and Hefter (4) and Goldschmidt and Strock (5) for selenium in certain genetic varieties of rocks and minerals, particularly sulphides, are summarized in table 9, in which the results for phosphates (table 6) are also included for convenience in comparison.

According to the available data (table 8), phosphate rock from deposits east of the Mississippi River contains less selenium than does that from deposits in the West. Aside from a crude sulphur from Colorado showing 8,350 p. p. m., pyrite, as would be expected, contained the largest quantities of selenium. The selenium content of phosphate rock from the western division of the United States agrees very well with that of other sedimentary deposits in this region. In the ascending order of the average results, shale, chalk, Permian phosphate, and limestone lie between the values (9 and 20 p. p. m., respectively) for ironstone (and mudstone) and bentonite.

TABLE 8.—*Selenium in rocks, minerals, soils, and waters of the United States west and east of the Mississippi River*

Location and material	Samples analyzed	Selenium	
		Range	Average
West of Mississippi River:	<i>Number</i>	<i>P. p. m.</i>	<i>P. p. m.</i>
Phosphate rock (Permian only).....	24	<0.8-85	<17.2
Limestone.....	6	1.5-46	19
Sandstone.....	14
Chalk (Niobrara).....	17	6.0-30	15
Gypsum.....	4	1.0-10	4.6
Gypsum sand.....	12
Sulphur (crude from Colorado).....	1	8,350
Pyrite.....	6	5.0-320	123
Ironstone and mudstone.....	4	4.0-14	9.0
Shale.....	80	.0-103	11
Bentonite.....	6	2.0-76	20
Soil.....	446	.0-41	2.0
Well water.....	12	.02-.2	.06
Creek and drainage water.....	6	.00-1.2	.2
East of Mississippi River:			
Phosphate rock.....	25	<.8-16	<2.7
Apatite.....	1	<.8
Pyrite.....	20	.0-250	70
Marcasite (from clay).....	2	.3-.6	4.5
Pyrrhotite.....	1	5.0
Chalcopyrite.....	1	10
Mispickel.....	1	(¹)
Shale.....	5	.15-.6	.25
Clay bed.....	6	.0-.4	.18

¹ Trace.² Samples of surface soil, sand, and clay.TABLE 9.—*Selenium in certain genetic varieties of minerals and rocks, nitrate beds, and waters*

Material	Samples analyzed	Selenium	
		Range	Average
	<i>Number</i>	<i>P. p. m.</i>	<i>P. p. m.</i>
Meteorites (iron, troilite, and chondite).....	5	1.5-132	47
Sulphides:			
Primary magmatic ¹	7	17-65	44
Pneumatolytic and hydrothermal ²	12	.8-190	29
From sedimentary deposits ³	13	.1-32	12
Oxides of iron and manganese ⁴	5	.5-5.4	2.0
Phosphates:			
Primary deposits.....	41	<.8-55	<10.9
Secondary deposits.....	34	<.8-16	<2.0
Limestone, Devonian.....	(⁵)1
Argillaceous-arenaceous rocks.....	(⁶)6
Chilean nitrate beds, caliche.....	(⁷)	5.2
Water from North Sea.....	2	.0033-.0044	.0038
Water from La Roche-Posay.....	32

¹ Pyrrhotite from Germany, Norway, South Africa, and Ontario, Canada.² From Norway, Germany, England, California, and Colorado.³ 1 sample from Newfoundland, others from Germany.⁴ 1 sample was a deep-sea nodule of manganese ore, others from Germany, Finland, and Newfoundland.⁵ Results from table 6.⁶ Composite sample representative of 32 limestones of Germany.⁷ Composite sample representative of deposits in Germany and Japan.⁸ Composite sample representative of 14 localities.⁹ Analyses by Taboury (#3).

When all the data are considered, it appears that sedimentary deposits, in general, carry less selenium than other deposits. Furthermore, larger quantities of selenium occur in primary than in secondary deposits.

SELENIUM IN SUPERPHOSPHATE AND PHOSPHORIC ACID

In view of the occurrence of selenium in phosphate rock in amounts ranging from <0.1 to 55 p. p. m. (tables 2 and 3), notable quantities of this element would ordinarily be expected in superphosphate and phosphoric acid manufactured from rock from certain phosphate deposits. Furthermore, selenium may be introduced into superphosphate and phosphoric acid as an impurity in the sulphuric acid used to decompose the rock. Considerable quantities of selenium are often present in sulphuric acid manufactured from pyrite or produced as a byproduct of the smelting of other sulphide ores; acid from these sources is used extensively in the fertilizer industry, particularly in the manufacture of superphosphate. Stoklasa (22) reported 26 to 58 p. p. m. of selenium in Glover tower acid and 15 to 42 p. p. m. in chamber acid. A sample of byproduct sulphuric acid (60° B.) from a copper-smelting operation in Tennessee contained 52 p. p. m. of selenium (6). The presence of selenium in sulphuric acid has also been noted by others (20, 24). Inasmuch as selenium occurs in coal (12) and also in coke (21), phosphoric acid produced by the electric-furnace and blast-furnace processes, particularly the latter, may also carry selenium as the result of using coke as a reducing agent and as fuel.

Stoklasa (22) found 15 to 36 p. p. m. of selenium in European superphosphate. Much smaller quantities were found in American superphosphates by the authors. The results (table 10) range from <0.8 to 4.0 p. p. m. As the samples were typical commercial superphosphates recently made from phosphate rock representing the principal deposits in this country, it appears that these results may be regarded as a fairly accurate range for the selenium content of superphosphate manufactured in the United States at the present time.

TABLE 10.—Selenium in superphosphate and phosphoric acid

ORDINARY SUPERPHOSPHATE

Sample no.	Type or source of phosphate rock	Source of sulphuric acid	P ₂ O ₅	Se
			Percent	P. p. m.
1315	Florida land pebble.....	Pyrite.....	19.20	1.5
1370	do.....	do.....	21.06	.8
1316	Tennessee brown rock.....	Sulphur.....	18.86	<.8
1414	Florida land pebble.....	Sludge acid from petroleum refining.....	17.85	<.8
1402	do.....	do.....	20.60	1.0

DOUBLE SUPERPHOSPHATE

1337	Florida land pebble.....	Sulphur.....	46.21	<0.8
1362	Tennessee brown rock.....	(1)	48.37	1.5
1372	Idaho.....	Copper-smelting operation in Montana.....	47.33	4.0

CRUDE PHOSPHORIC ACID

1200	Idaho.....	Copper-smelting operation in Montana.....	20.26	<0.07
1199	do.....	do.....	37.80	<.07
1058	Tennessee brown rock.....	Copper-smelting operation in Tennessee ¹	16.04	.14
1057	do.....	do.....	41.38	.5

¹ Phosphoric acid manufactured by the blast-furnace process was used.² A sample of acid (60° B.) from this smelting operation contained 52 p. p. m. of selenium (6).

The results in table 10 present a few additional points of special interest. Only a very small part of the selenium in the raw materials was found in the superphosphate or phosphoric acid. For example, the phosphoric acids produced from Idaho phosphate by the sulphuric-acid process carried less than 0.07 p. p. m. of selenium, despite the fact that the rock (Conda, Idaho) contains 8 to 40 p. p. m. (table 2) and the copper ore from which the sulphuric acid was produced as a by-product was probably also seleniferous. On processing phosphate rock from Conda, Idaho, it is the practice to calcine the rock at about 700° C. (14), in order to eliminate most of the organic matter which otherwise would seriously foul the acid and apparatus. At least a portion of the selenium of the phosphate rock is probably volatilized by this treatment.⁶ Doubtless a part of any selenium that is present in the mixture of rock and sulphuric acid remains with the sludge and is thus eliminated from the phosphoric acid. Furthermore, selenium may be lost by volatilization during concentration operations, although such a loss is not indicated by the results for the Tennessee acids. It may also be noted in this connection that, whereas a sample of sulphuric acid from the same source as that used in the manufacture of the Tennessee brown-rock acids carried 52 p. p. m. of selenium, the concentrated phosphoric acid contained only 0.5 p. p. m. and the dilute acid considerably less. Therefore, it would appear that, under the conditions of manufacture in this country, only a small part of the selenium occurring in the raw materials, in general, finds its way into the finished phosphate products.

SUMMARY

Results are given for selenium in 96 samples of phosphate rock and 3 samples of apatite from various deposits of the world, 8 representative samples of commercial superphosphates manufactured from domestic rock, and 4 samples of crude phosphoric acid produced by the sulphuric-acid process.

The results for selenium in natural phosphates range from <0.1 p. p. m. in a Tennessee brown rock to 55 p. p. m. in Wyoming and Algerian phosphates. The occurrence of selenium in natural phosphates is discussed from the following points of view: (1) Selenium-bearing constituents; (2) primary and secondary deposits; (3) geologic age of deposits; and (4) a comparison of phosphates with other geologic formations. Accordingly, the data indicate (1) that organic matter and, to a less extent, inorganic sulphides are important carriers of selenium in phosphate rock, (2) that primary deposits are in general richer in this element than are secondary deposits, (3) that deposits belonging to the Permian and Cretaceous ages contain the most selenium, and (4) that the selenium content of phosphate deposits is about the same as that of other sedimentary deposits in the same region.

The quantity of selenium in superphosphate ranges from <0.8 to 4.0 p. p. m., and in phosphoric acid is 0.5 p. p. m. or less. According to the available data only a small fraction of the selenium occurring in the natural materials from which superphosphate and phosphoric acid are made finds its way into the finished product.

⁶ According to the results of a single experiment with Wyoming rock no. 948, a highly seleniferous phosphate (table 2), selenium was completely volatilized when the phosphate was calcined in the presence of water vapor at 1,400° C. for 30 minutes.

LITERATURE CITED

- (1) ALEXANDER, L. T., and JACOB, K. D.
1930. MECHANICAL ANALYSIS OF FINELY DIVIDED NATURAL PHOSPHATES. U. S. Dept. Agr. Tech. Bull. 212, 24 pp., illus.
- (2) BLACKWELDER, E.
1915. ORIGIN OF THE ROCKY MOUNTAIN PHOSPHATE DEPOSITS. (Abstract) Geol. Soc. Amer. 26: 100-101.
- (3) BYERS, H. G.
1935. SELENIUM OCCURRENCE IN CERTAIN SOILS OF THE UNITED STATES WITH A DISCUSSION OF RELATED TOPICS. U. S. Dept. Agr. Tech. Bull. 482, 48 pp., illus.
- (4) GOLDSCHMIDT, V. M., and HEFTER, O.
1933. ZUR GEOCHEMIE DES SELENS. Nachr. Gesell. Wiss. Göttingen, Math. Phys. Kl. 2: [245]-252.
- (5) ——— and STROCK, L. W.
1935. ZUR GEOCHEMIE DES SELENS II. Nachr. Gesell. Wiss. Göttingen, Math. Phys. Kl. (n. s.) 1: 123-142.
- (6) HILL, W. L., MARSHALL, H. L., and JACOB, K. D.
1932. COMPOSITION OF CRUDE PHOSPHORIC ACID PREPARED BY SULFURIC ACID PROCESS. Indus. and Engin. Chem. 24: 1064-1068, illus.
- (7) HURD-KARRER, A. M.
1933. INHIBITION OF SELENIUM INJURY TO WHEAT PLANTS BY SULFUR. Science (n. s.) 78: 560.
- (8) ———
1934. SELENIUM INJURY TO WHEAT PLANTS AND ITS INHIBITION BY SULPHUR. Jour. Agr. Research 49: 343-357, illus.
- (9) ———
1935. FACTORS AFFECTING THE ABSORPTION OF SELENIUM FROM SOILS BY PLANTS. Jour. Agr. Research 50: 413-427, illus.
- (10) INTERNATIONAL CONGRESS OF GEOLOGISTS.
1928. LES RÉSERVES MONDIALES EN PHOSPHATES. Bur. 14th Cong. Geol. Internatl., Espagne, 1926. 2 v., illus. Madrid.
- (11) JACOB, K. D., HILL, W. L., MARSHALL, H. L., and REYNOLDS, D. S.
1933. THE COMPOSITION AND DISTRIBUTION OF PHOSPHATE ROCK WITH SPECIAL REFERENCE TO THE UNITED STATES. U. S. Dept. Agr. Tech. Bull. 364, 90 pp.
- (12) JORISSEN, A.
1896. SUR LA PRÉSENCE DU MOLYBDÉNE, DU SELENIUM, DU BISMUTH, ETC., DANS LE TERRAIN HOUILLE DU PAYS DE LIÉGE. Ann. Soc. Geol. Belg. (1895-96) 23: [101]-105.
- (13) KNIGHT, H. G.
1935. THE SELENIUM PROBLEM. Jour. Assoc. Off. Agr. Chem. 18: 103-108.
- (14) LARISON, E. L.
1929. MANUFACTURE OF HIGH-ANALYSIS PHOSPHATES. Indus. and Engin. Chem. 21: 1172-1175, illus.
- (15) MANSFIELD, G. R., and GIRTY, G. H.
1927. GEOGRAPHY, GEOLOGY, AND MINERAL RESOURCES OF PART OF SOUTHEASTERN IDAHO . . . WITH DESCRIPTIONS OF THE CARBONIFEROUS AND TRIASSIC FOSSILS. U. S. Geol. Survey Prof. Paper 152, 453 pp., illus.
- (16) MARSHALL, H. L.
1934. THE OCCURRENCE OF FERROUS IRON IN PHOSPHATE ROCK. Jour. Agr. Research 49: 71-76.
- (17) MELLOR, J. W.
1930. SELENIUM. In his A Comprehensive Treatise on Inorganic and Theoretical Chemistry, v. 10, ch. 58, illus. London, New York, [etc.].
- (18) NELSON, E. M., HURD-KARRER, A. M., and ROBINSON, W. O.
1933. SELENIUM AS AN INSECTICIDE. Science (n. s.) 78: 124.
- (19) ROBINSON, W. O., DUDLEY, H. C., WILLIAMS, K. T., and BYERS, H. G.
1934. DETERMINATION OF SELENIUM AND ARSENIC BY DISTILLATION IN PYRITES, SHALES, SOILS, AND AGRICULTURAL PRODUCTS. Indus. and Engin. Chem., Analyt. Ed. 6: 274-276, illus.
- (20) SCHULZ, F.
1911. ÜBER DEN EINFLUSS VON SELEN BEI DER RAFFINATION DER MINERALÖLE. Chem. Ztg. 35: 1129-1130.

- (21) SMITH, J. F.
1903. NOTE ON SELENIUM IN COKE. *Jour. Soc. Chem. Indus.* 22: 201.
- (22) STOKLASA, J.
1922. ÜBER DIE EINWIRKUNG DES SELENS AUF DEN BAU- UND BETRIEB-STOFFWECHSEL DER PFLANZE BEI ANWESENHEIT DER RADIOAKTIVITÄT DER LUFT UND DES BODENS. *Biochem. Ztschr.* 130: [604]-643, illus.
- (23) TABOURY, F.
1909. SUR LA PRÉSENCE DU SÉLÉNIUM DANS LES EAUX MINÉRALES DE LA ROCHE-POSAY [VIENNE]. *Bull. Soc. Chim. France* (4) 5: 865-867.
- (24) VAVERKA, A.
1924. SUR LE DOSAGE DES COMPOSÉS AZOTÉS DANS L'ACIDE SULFURIQUE ET SUR LA COLORATION DE L'ACIDE DE GLOVER. *Indus. Chim. [Paris]* 11: 253-255.
- (25) WILLIAMS, K. T., and BYERS, H. G.
1934. OCCURRENCE OF SELENIUM IN PYRITES. *Indus. and Engin. Chem., Analyt. Ed.* 6: 296-297.

ROOT SYSTEMS OF CERTAIN TREES AND SHRUBS GROWN ON PRAIRIE SOILS¹

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INTRODUCTION

Actual data on the depth and spread of tree roots are not abundant, and such as there are deal mainly with fruit trees grown under fairly humid conditions. The study here described was made to determine the depth and spread of certain forest trees and shrubs growing in North Dakota. The work was begun in the autumn of 1934.

REVIEW OF LITERATURE

Zon (20)³ states that the water table under the forest is lower than that outside, for the forest through transpiration consumes more water than any other cover crop, and draws the water from a greater depth. Halden (7) says that in the sterile zone on the borders of forest stands on south and west exposures the soil is drier than either inside or outside of the groves, and that this dryness is more pronounced on sand than on clay.

Goff (6), Ballantyne (1), Oskamp and Batjer (11), Partridge and Veatch (12), Clark (3), Goff (5), Rogers (13), and Mason (10) found the greater part of the roots of fruit trees in the upper 3 feet of soil and the maximum depth of penetration 9 feet.

Holch (8) stated that the greatest depth to which the roots of young forest seedlings penetrated during the first year was 5.7 feet.

Laitakari (9), in a study of Scotch pine (*Pinus sylvestris*), found that root systems extended far beyond the spread of branches, with a maximum depth of 3 meters and that spruce had a still shallower root system. Cheyney (2) found that jack pine also had a shallow root system, and Woodroof and Woodroof (19) reported that roots of pecan in Georgia rarely reach a depth of more than 5 feet.

Weaver and Kramer (18), studying oak (*Quercus macrocarpa*) in Nebraska, found roots with 2 to 4 times the radial spread of the branches. Deep roots reached 14 feet.

The foregoing observations hardly seem to support the common belief expressed by Zon (21) and Shepard (13) that tree roots penetrate to great depths.

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² Indebtedness is acknowledged to the Federal Transient Bureau of Fargo for supplying transients to assist in the work, and to the transients themselves for their splendid cooperation; to Marvel Lien who supervised the work; and to Prof. C. B. Waldron who supplied the information on the ages of the plantations.

³ Reference is made by number (italic) to Literature Cited, p. 1091.

LOCATION AND MATERIALS

Most of the studies here recorded were made on the grounds of the North Dakota Agricultural College and Agricultural Experiment Station at Fargo. The soil is a Fargo clay, such as characterizes parts of the Red River Valley. It is very level and is sometimes deficient in drainage during the early spring months. The black surface soil extends downward to a depth of about 3 feet, where it changes to a light-colored calcareous, clayey subsoil. There is no true hardpan and no stratum of rock. Because of the continued deficiency of rainfall for many years the soil has become very dry and the water table is nowhere higher than 15 feet.

To determine the behavior of tree roots under different soil conditions, a snow trap planted by the Northern Pacific Railway Co. along its right-of-way was also studied. This planting, 25 years old, is located 38 miles west of Fargo, just outside the Red River Valley, in Barnes loam, a light soil.

METHODS

In general, the following method was employed in making these studies: Where there was a row of trees, as there was in most cases, a trench 18 inches to 2 feet in width was dug parallel to the row and under the outer edge of the branches of the trees. The digging was deep enough to sever all roots. After the trench was dug the upper 1 foot was separated from the remainder by a cord stretched parallel to the ground surface, and the roots which came through the trench wall in this area were counted and classified according to diameter. After this was done, the second foot layer was handled in the same way, then the third, the fourth, and so on until a depth was reached where no more roots were found. Later the larger roots which crossed the trench were dug out. They were traced from the point where they left the trench until they became too small to follow.

In cases where it was thought that root penetration might be deeper beneath the tree than farther from it, excavations were made directly under the tree. When it was suspected that the first specimens might not represent a fair sample of the species, and other plants were available, these were studied also. By such means the depth and distribution of the root systems in the various soil levels were ascertained, and the maximum spread of the roots in the soil and their maximum depths were determined and tabulated. In addition, a limited number of soil-moisture determinations were made.

PRESENTATION OF DATA

ROOT DISTRIBUTION IN FARGO CLAY AND BARNES LOAM

Table 1 shows a compilation of data secured from root studies with 31 species of trees and shrubs growing on Fargo clay at Fargo, N. Dak., without irrigation. Trenches made in studying these species ranged from 20 feet to 600 feet in length for each species. According to the United States Weather Bureau records, the mean normal rainfall at Fargo is 22.34 inches per year, but for the past 15 years the average has been only 17.78 inches and for the past 6 years, 15.43 inches.

TABLE 1.—Depth of penetration and lateral spread of tree and shrub roots in Fargo clay

[illegible]

The data in table 1 show that 97.3 percent of the roots growing in Fargo clay were confined to the upper 4 feet of soil. The deepest penetration of any root—that of Hibernial apple—was 10½ feet, and the slightest—that of butternut—was 2½ feet. The average of all species gave a mean maximum root length of 1.3 times the tree height. Blue spruce (with 0.4) among the evergreens, and *Tamarix pentandra* (with 0.7) among the deciduous trees had the smallest relative spread; while jack pine (with 1.9) among the evergreens, and chokecherry and black walnut (with 2.1 each) among the deciduous plants had the largest, (fig. 1, B-O).

Ten Eyck (15) found, in a study of the root systems of cultivated plants grown at this station in the same type of soil, that Rural

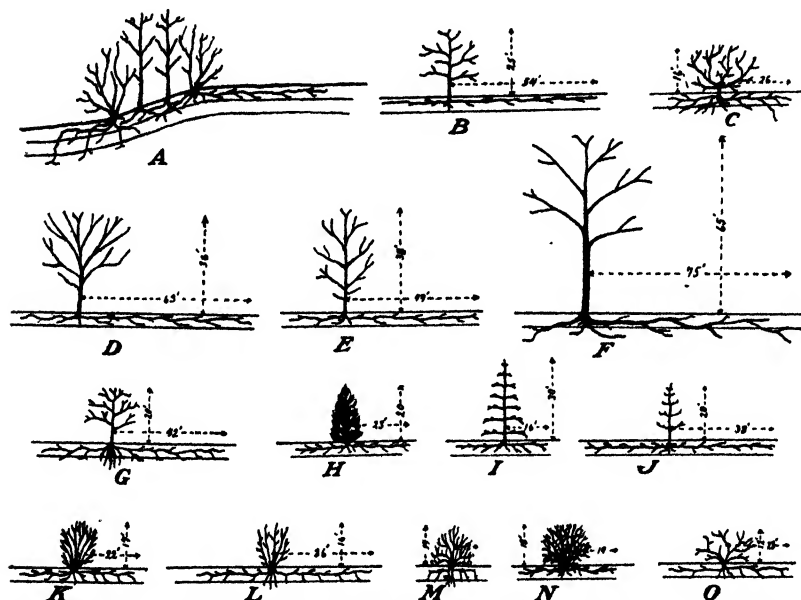


FIGURE 1.—Diagram showing distribution of tree and shrub roots in Fargo clay. (In the case of wide-spreading plants, roots are shown fully in one direction only. Lines beneath the soil level indicate 5-foot depths). A, cross section of four tree rows, on a slope from a dry area into a moist one. Two rows of green ash grow in the middle with one row of golden willow on each side. Note the wider spread and shallower rooting of willow on the dry soil as compared to that on the soil which in normal years is flooded each spring. B, black walnut, *Juglans nigra*. C, hibernial apple, *Malus sylvestris*. D, American elm, *Ulmus americana*. E, green ash, *Fraxinus lanceolata*. F, northern cottonwood, *Populus monilifera*. G, mossycup oak, *Quercus macrocarpa*. H, Colorado juniper, *Juniperus scopulorum*. I, Colorado spruce, *Picea pungens*. J, jack pine, *Pinus banksiana*. K, Siberian pea-tree, *Caragana arborea*. L, Chokecherry, *Prunus virginiana*. M, Amur tamarix, *Tamarix pentandra amurensis*. N, buck thorn, *Rhamnus cathartica*. O, Silver buffaloberry, *Shepherdia argentea*.

potato roots penetrated 3 feet deep, sugar beets 3½ feet, native grasses 4 feet, flax 2½ feet, and *Bromus inermis* at least 5½ feet and probably more. Weaver (16), in Nebraska, found that wild alfalfa penetrated 16½ feet, buffalo grass 7 feet, wheat 3 feet, corn 8 feet, and alfalfa 20 feet. Horseradish is reported (17) as penetrating 14 feet deep, carrots 8 feet, and squash as having a 17-foot spread of roots with a 6-foot downward penetration. Thus, it would appear that tree roots occupy much the same soil layers as do ordinary field crops.

To compare the results obtained on the heavy clay soil with uncertain drainage, characteristic of the conditions at Fargo, another

series of observations was made near Buffalo, N. Dak., 38 miles west of Fargo. Buffalo lies just outside the Red River Valley, and the soil, a Barnes loam, is lighter and more sandy, and except for the greater organic content near the surface, is uniform far below any depth reached in the Fargo studies. Trees of the following species were excavated and studied: Golden willow, green ash, northern cottonwood, boxelder, and soft maple. It was found that the depth of penetration and the spread of the roots in this soil were practically the same as in the heavy soil; perhaps a few inches deeper, but not enough to affect the general conclusion. Data on golden willow and green ash are given in table 2.

TABLE 2.—*Effect of soil type and moisture on the roots of golden willow and green ash*

Foot of depth and diameter of root (inches)	Roots in 50-foot trench							
	Golden willow				Green ash			
	Sandy soil		Heavy clay soil		Sandy soil		Heavy clay soil	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
First:	Number	Number	Number	Number	Number	Number	Number	Number
0-14.....	290	83	92	64	105	252	60	70
14-14.....	3	10	25	12	0	28	9	8
14-1.....	3	4	2	0	20	8	9	0
1-2.....	0	0	4	0	5	8	0	2
Above 2.....	3	2	0	4	0	16	0	2
Second:								
0-14.....	135	94	118	96	170	164	72	93
14-14.....	6	6	22	20	5	16	6	15
14-1.....	3	0	10	12	5	8	15	4
1-2.....	0	0	0	4	0	4	3	2
Above 2.....	0	4	0	0	0	12	0	0
Third:								
0-14.....	54	176	92	104	145	152	111	260
14-14.....	0	6	10	4	0	8	9	6
14-1.....	0	2	4	4	0	0	6	6
1-2.....	0	0	0	0	0	0	0	2
Above 2.....	0	0	0	0	0	4	0	2
Fourth:								
0-14.....	27	134	53	136	55	116	52	35
14-14.....	0	2	0	12	0	0	0	6
14-1.....	3	4	0	0	0	0	0	1
1-2.....	0	0	0	0	0	0	0	0
Above 2.....	0	4	0	0	0	0	0	0
Fifth:								
0-14.....	24	64	0	84	0	28	0	75
14-14.....	0	0	0	4	0	0	0	4
Sixth:								
0-14.....	0	94	0	92				
Seventh:								
0-14.....	0	28	0	24				
14-14.....	0	0	0	8				
Eighth:								
0-14.....	0	6	0	0				
Ninth:								
0-14.....	0	2	0	0				
Tenth:								
0-14.....	0	2	0	0				

Maximum depth reached by traced roots of golden willow in heavy clay soil: Dry 6 feet 8 inches; wet 14 feet.

EFFECT OF SOIL MOISTURE ON ROOTS

Around the edge of the tree planting at Fargo there is a slough which is dry during and greater part of the year, but during the spring of most years water stands in it for such long periods that it

is not suitable for ordinary crops. Four rows of trees, spaced 10 feet apart, have been planted at the edge of the wet area as a windbreak for the horticultural plots. The 2 middle rows are green ash and the 2 outside rows are golden willow. Excavations were made in the low area and the distribution of roots growing under these moist conditions were compared with those on higher ground where the only moisture available was from the rain that fell there.

In the case of the site near Buffalo, although the soil profile is relatively uniform throughout, the surface contour is somewhat irregular; that is, there are places into which the water from melting snow and flood water runs each spring, and other high points from which any excess surface water runs off. Excavations were made both on these high points and in the low places, and the roots of such trees as were available were studied. Table 2 presents the findings with respect to golden willow and green ash under the two moisture conditions.

Table 2 shows that the willow rooted very much more deeply in both the sandy soil and clay soil when extra moisture was available. Figure 1, A, shows a diagram of a cross section of the windbreak at Fargo running from the high land into the flooded area. This diagram illustrates the difference in depth of rooting under the two conditions and also the much greater spread of the roots near the surface where the soil is dry. These deep roots of the willow were found to penetrate almost straight down until they reached their maximum depth, where they spread out abruptly. Moisture studies showed that at this point, where a 7-foot layer of hard shale changed to a softer layer, there was 10 percent more moisture than at the top of the layer of shale.

Table 2 also shows the difference in the rooting of green ash under the two conditions of soil moisture. Here, again, it will be noted that where the soil receives excess water the roots penetrate deeper, although they do not reach the depth attained by those of the willow. A similar condition was found with respect to cottonwood, soft maple, and boxelder in the two locations on sandy soil. Trees of these species were not available for a similar comparison in the heavy soil. This deeper penetration where extra water is available corresponds closely to the finding of Weaver (16) that wheat roots grown without irrigation penetrate to a depth of 3 feet and where irrigated to 5 feet, and that rye growing in dry ground penetrates to 5 feet and in moist soil to 8 feet. Shallow wide-spread root systems in semiarid regions permit trees to utilize deficient natural rainfall more fully since, as reported by Finnell (4), little of the water reaches the subsoil.

These root studies indicate that many of the trees which are known to be none too resistant to drought have a greater tendency to form deep, penetrating vertical roots in moist locations than do most of the drought-resistant forms. Soft maple produces deep vertical roots occasionally. Cottonwood and Northwest poplar (a supposedly hybrid species) both form such roots occasionally though none of these produce a deep-penetrating root system in anything like the profusion that the golden willow, bronze golden willow, and white willow do when moisture is abundant. Known drought-resistant trees such as oak and choke cherry, which have comparatively deep root

systems, differ from those without such drought resistance in that the deep roots are characteristic of these two species regardless of moisture conditions.

SUMMARY

At Fargo, N. Dak., which has an average rainfall of 22.34 inches, a study involving 31 species of trees and shrubs showed more than 97 percent by number of the roots to be in the first 4 feet of soil.

Without irrigation, and if permitted to spread, the roots extend horizontally from 0.4 to 2.1 times the height of the tree, depending upon the species.

When additional water, but not an excessive quantity is supplied, tree roots spread less and penetrate deeper. The effect of the additional moisture is greater on some species than on others.

The general distribution of the tree roots was much the same in clay as in sandy soils.

LITERATURE CITED

- (1) BALLANTYNE, A. B.
1916. FRUIT TREE ROOT SYSTEMS SPREAD AND DEPTH AS PARTLY DETERMINED BY EXCAVATIONS ON THE SOUTHERN EXPERIMENT FARM, ST. GEORGE, UTAH. *Utah Agr. Expt. Sta. Bull.* 143, 15 pp., illus.
- (2) CHEYNEY, E. G.
1932. THE ROOTS OF A JACK PINE TREE. *Jour. Forestry* 30: 929-935, illus.
- (3) CLARK, E. R.
1934. ROOT PENETRATION OF NINE MATURE FRUIT TREES ON HEAVY SILT LOAM SOILS. *Okla. Panhandle Agr. Expt. Sta. Bull.* 55: 13-16.
- (4) FINNELL, H. H.
1929. HEAVY PLAINS SOIL MOISTURE PROBLEMS. *Okla. Agr. Expt. Sta. Bull.* 193, [7] pp.
- (5) GOFF, E. S.
1887. OBSERVATIONS ON ROOT GROWTH. *N. Y. Agr. Expt. Sta. Ann. Rept.* (1886) 5: 157-165.
- (6) ———
1897. A STUDY OF THE ROOTS OF CERTAIN PERENNIAL PLANTS. *Wis. Agr. Expt. Sta. Ann. Rept.* 14: 286-298, illus.
- (7) HALDEN, B. E.
1932. MARKTORKAN Å SAND-ÖCH GRUSMARKET. *Skogsvårdsfor. Tidskr.* 30: 39-131, illus. [In Swedish. German summary, pp. 122-131.]
- (8) HOLCH, A. E.
1931. DEVELOPMENT OF ROOTS AND SHOOTS OF CERTAIN DECIDUOUS TREE SEEDLINGS IN DIFFERENT FOREST SITES. *Ecology* 12: 259-298, illus.
- (9) LAITAKARI, E.
1927. MÄNNYN JUURISTO. MORFOLOGINEN TUTKIMUS. (THE ROOT SYSTEM OF PINUS [PINUS SYLVESTRIS], A MORPHOLOGICAL INVESTIGATION.) *Acta Forest. Fennica* 33, no. 1, 380 pp., illus. [In Finnish. Summary in English, pp. 307-380.]
- (10) MASON, S. C.
1911. DROUGHT RESISTANCE OF THE OLIVE IN THE SOUTHWEST STATES. *U. S. Dept. Agr., Bur. Plant Indus. Bull.* 192, 60 pp., illus.
- (11) OSKAMP, J., and BATJER, L. P.
1932. SOILS IN RELATION TO FRUIT GROWING IN NEW YORK. PART II. SIZE, PRODUCTION, AND ROOTING HABIT OF APPLE TREES ON DIFFERENT SOIL TYPES IN THE HILTON AND MORTON AREAS, MONROE COUNTY. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 550, 45 pp., illus.
- (12) PARTRIDGE, N. L., and VEATCH, J. O.
1932. THE RELATIONSHIP BETWEEN SOIL PROFILE AND ROOT DEVELOPMENT OF FRUIT TREES. *Mich. Agr. Expt. Sta. Quart. Bull.* 14: 200-207.

- (13) ROGERS, W. S.
1933. ROOT STUDIES. III. PEAR, GOOSEBERRY AND BLACK CURRANT ROOT SYSTEMS UNDER DIFFERENT SOIL FERTILITY CONDITIONS, WITH SOME OBSERVATIONS ON ROOT STOCK AND SCION EFFECT IN PEARS. *Jour. Pomol. and Hort. Sci.* 11: 1-18, illus.
- (14) SHEPARD, W.
1928. FORESTS AND FLOODS. U. S. Dept. Agr. Circ. 19; 24 pp., illus.
- (15) TEN EYCK, A. M.
1900. A STUDY OF THE ROOT SYSTEMS OF CULTIVATED PLANTS GROWN AS FARM CROPS. N. Dak. Agr. Expt. Sta. Bull. 43, pp. 535-550, illus.
- (16) WEAVER, J. E.
1926. ROOT DEVELOPMENT OF FIELD CROPS. 291 pp., illus. New York.
- (17) ——— and BRUNER, W. E.
1927. ROOT DEVELOPMENT OF VEGETABLE CROPS. 351 pp., illus. New York.
- (18) ——— and KRAMER, J.
1932. ROOT SYSTEM OF QUERCUS MACROCARPA IN RELATION TO THE INVASION OF PRAIRIE. *Bot. Gaz.* 94: 51-85, illus.
- (19) WOODROOF, J. G., and WOODROOF, N. C.
1934. PECAN ROOT GROWTH AND DEVELOPMENT. *Jour. Agr. Research* 49: 511-530, illus.
- (20) ZON, R.
1927. FORESTS AND WATER IN THE LIGHT OF SCIENTIFIC INVESTIGATION. 106 pp., illus. Washington. (Reprinted from 62d Cong., 2d sess., S. Doc. 469).
- (21) ———
1934. THE SHELTERBELT PROJECT. N. and S. Dak. Hort. 7(1):139.

GERMINATION STUDIES ON AGED AND INJURED SEEDS¹

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INTRODUCTION

From 1909 to 1933, various investigations upon problems of germination were carried on at the North Dakota Seed Laboratory by the writer and his assistants and by advanced students under the writer's supervision. The present paper includes results of some of the experiments that deal with special conditions of seeds.

CLOVER AND ALFALFA

LONGEVITY OF NORMAL SAMPLES

Some of the first studies dealt with the seeding value of "hard" seeds in clovers. Much has been published on this subject since the results of these studies were reported, and only certain features need to be considered here. In one of the first experiments six samples of alfalfa (*Medicago sativa* L.) and two each of sweetclover (*Melilotus alba* Desr.) and red clover (*Trifolium pratense* L.) were tested every 2 months for hard-seed content. These samples, assembled in the early winter of 1913-14, have been continued as a longevity trial, and 20 years' data are now available. The samples were kept in ordinary manila seed envelopes, stored in ventilated galvanized boxes which were kept in the laboratory either on or near the floor. Germination tests were made between blotters by standard methods, and 500 seeds were used for each test during the first season.

The samples for the trials were selected with much care. The first three of alfalfa shown in table 1 were grown in western North Dakota in 1913, no. 14497 was grown in Montana, and no. 14469 in South Dakota. No. 14487 was imported Turkestan seed which came from a wholesale seed house and showed the typical color and intermixture of foreign seeds. Both lots of sweetclover were secured from seed houses, the crop being new at that time and no locally grown seed available. No. 14295 was from a St. Louis dealer and no. 15093 was said to be Kansas-grown. No. 14295 was rather immature and contained after recleaning, about 30 percent of greenish-colored seeds. Separate tests showed a germination of 38 percent and 29 hard for these green seeds as compared with 66 and 33 hard for the yellow. The red clovers were grown in Cass and Traill Counties, respectively, in North Dakota. A note in the original entry states: "All lots containing trash and light, shrunken seeds were blown in the vertical air blast separator to grade them evenly." Notwithstanding this, the samples are not quite so carefully prepared as would be desired by present standards.

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TABLE 1.—*Germination and percentage of hard seed in various samples of alfalfa, sweetclover, and red clover seed during the first year of storage*

Sample no.	Percentage of germination and of hard seed in—											
	February		April		June		August		October		December	
	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed
Alfalfa:												
14358-----	64	33	62	33	77	21	76	21	81	15	89	8
14397-----	59	38	59	33	57	27	66	28	79	18	81	14
14407-----	67	32	67	27	78	19	79	20	84	12	84	12
14497-----	79	16	79	16	83	14	85	13	87	10	91	7
14409-----	96	3	91	6	84	5	96	3	96	3	96	2
14487-----	95	4	95	2	93	3	96	2	95	1	94	3
Sweetclover:												
14298-----	59	35	57	33	58	31	64	23	60	20	63	25
15093-----	68	27	70	26	69	26	69	26	71	22	69	27
Red clover:												
14448-----	62	35	57	36	67	29	71	22	69	26	69	28
14448-----	83	12	83	8	91	7	90	7	91	6	92	4

After the first year, only 200 seeds were used in each test as it was felt that the variations would be somewhat smaller and that these samples would give the significant differences. In general, differences of less than 5 percent are not considered significant unless they appear consistently through a considerable number of tests. The causes and extent of variation have been discussed by the writer in another paper (8).²

Germination tests, although carried out under standard conditions in standard chambers, are subject to some variations, especially in respect to moisture. It is believed that the common legume seeds are as little sensitive as any kinds of seeds and that this factor is relatively unimportant. The personal factor, however, is important in longevity trials. The actual work on these samples was done by six or more different workers as the laboratory staff changed from year to year, but at all times the tests were under the supervision of the writer. More important than the personal element is the fact that old seeds differ from new both in behavior and appearance. This makes it more difficult in the case of old seeds to determine "normal" germination, "hard" seeds, etc. Even if the work is done by the same person each year, his standards may change unconsciously. Seed laboratory methods, especially the interpretation of germination tests, have been under constant scrutiny in an effort to secure more nearly uniform procedure. Some soil tests of these samples have been made but not regularly, and a field trial of 4 of the alfalfas and 1 sweetclover is reported in connection with other trials (p. 1098). The foregoing discussion applies to the data in tables 1 and 2, and also to other trials to be described in later paragraphs.

A summary of the results of the germination tests with alfalfa, sweetclover, and red clover is given in table 1. It will be noted in table 1 that the alfalfa which began with a fairly high percentage of hard seed showed a well-marked decrease by June, and a still further decrease by late fall. A similar, but less marked effect, appears in the red clover. One sweetclover sample showed practically no change,

² Reference is made by number (italics) to Literature Cited, p. 1106.

while the other showed a decrease in hard seeds but without a corresponding increase in germination. A test of all samples in June 1915 following gave practically the same results as the December test. A summary by 5-year periods is shown in table 2.

TABLE 2.—*Germination and percentage of hard seed in various samples of alfalfa, sweetclover, and red clover seed by 5-year¹ intervals over a period of 20 years*

Sample no.	Percentage of germination and of hard seed in—									
	1914		1920		1924		1929		1934	
	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed
Alfalfa:										
14358.....	64	33	70	1	69	2	55	0	53	2
14397.....	59	38	72	3	68	3	71	3	64	4
14407.....	67	32	79	3	72	5	65	3	55	3
14497.....	79	16	53	1	59	5	53	2	54	1
14469.....	96	3	81	2	78	2	81	1	82	1
14487.....	95	4	95	0	86	0	85	0	75	0
Sweetclover:										
14295.....	59	35	32	30	28	23	22	21	26	18
15093.....	68	27	71	16	69	15	58	14	48	22
Red clover:										
14446.....	62	35	56	27	46	14	32	14	16	17
14448.....	83	12	40	13	39	4	17	3	7	6

¹ No test was made between 1914 and 1920.

Table 2 indicates (1) almost complete loss of hard seed in the alfalfa during the first 5 years, but a long retention of a small percentage; (2) retention of over 50 percent of viability in the same samples at 20 years; (3) retention of 75 to 80 percent of viability in two samples of alfalfa which contained practically no hard seeds at the beginning; (4) a much slower decrease of hard seeds and about equal decrease in germination in the sweetclover, the total loss being greater than in the alfalfa; (5) a still more rapid decline in red clover to a very low germination at 20 years; and (6) a marked difference in the behavior of different samples of the same kind of seed.

The finding of an exceptionally high retention of viability in two samples of alfalfa which had a low content of hard seed at the beginning of the experiment is perhaps surprising, and emphasizes the individuality of samples. Since the hard-seeded condition in alfalfa is rather temporary, there seems little reason to expect that it would affect the longevity. Where this condition is of long duration as in sweetclover, we naturally expect the permeable seeds to lose their vitality first, the hard seeds retaining theirs at least until the coats become permeable.

The first experiment had shown a marked decrease in hard seeds in alfalfa samples during the first year, but more frequent tests seemed desirable. A new series of samples which had been received from November 1914 to April 1915 was assembled and tested in the same way the 1st of each month from April to October. These were fresh seeds all with a high percentage of hard seeds and grown mostly in North Dakota (1 Minnesota, 2 Montana, 1 unknown). The averages of the results are given in table 3.

TABLE 3.—Germination of and percentage of hard seed in various samples of alfalfa, sweetclover, and red clover seed between April and October

Seed	Percentage of germination and of hard seed in—									
	April		May		June		July		October	
	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed	Germination	Hard seed
Alfalfa (8 samples).....	43	55	59	39	63	35	66	32	72	26
Sweetclover (3 samples).....	30	66	36	60	33	63	33	59	33	60
Red clover (3 samples).....	60	39	62	36	65	34	64	35	64	35

These results bear out those of the first trial, in showing a sharp decrease in the percentage of hard seeds in the alfalfa in early spring and a continued decrease at a slower rate during the rest of the summer. The sweetclover and red clover showed practically no change during the year. Retained for 15 years longer, the samples have

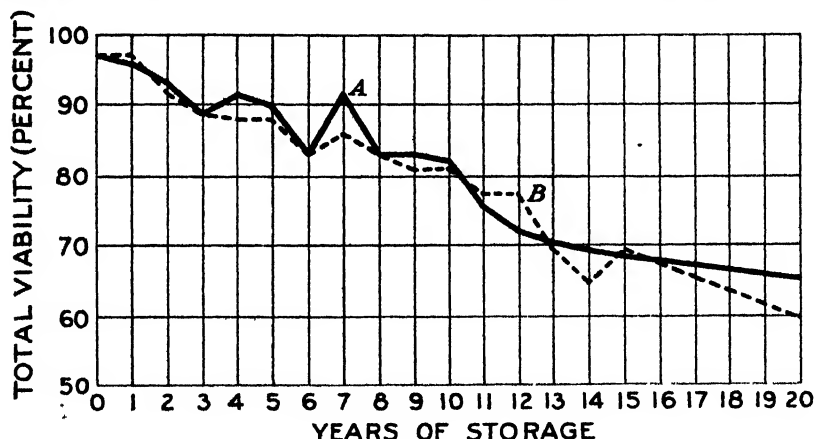


FIGURE 1.—Results of combined germination tests of all samples of alfalfa (A) and sweetclover (B) after various periods of storage. No data for the years 16 to 19, inclusive.

shown decreases comparable to those in table 2, again with considerable differences between apparently similar samples.

A third similar lot of samples was selected in 1920 for a scarified seed test. The unscarified portions of these samples were tested each year until 1930. By combining all the data available for the different lots of normal alfalfa and sweetclover seed, and considering only total viability (germinating plus hard), the graph shown in figure 1 was secured.

The results agree in general with those found by Sifton (7), but indicate considerably higher retention of viability. Possibly this is due to the fact that samples containing a high percentage of hard seeds were used for the most part. However, the highest germination of all samples at the age of 20 years was from two samples of alfalfa which contained very few hard seeds. Sifton has called attention to the rather sudden loss of vitality in the cereal seeds after about the tenth year, as compared with a gradual decline in the clovers. The

different behavior of clovers is possibly accounted for by the fact that they have a less uniform maturity, are subjected to a greater degree of injury in threshing, and undergo a gradual loss of impermeable seed coat.

LONGEVITY OF SCARIFIED SEEDS

The longevity of scarified sweetclover seed has been discussed quite fully in another paper (11, p. 13). The behavior of scarified alfalfa seed is essentially similar. The scarified seeds lose their vitality in the course of 1 to 2 years, probably as a result of increased oxidation permitted by the broken seed coats, and the loss of vitality in the remaining seeds of the sample then proceeds as with unscarified seeds. Different samples may behave quite differently owing to variations in degree of scarification or other abrasions in threshing, or to degree of maturity and other natural influences. The fact that the hard seeds of alfalfa become permeable to a large extent during the spring of the first year, and that they will germinate better in the field than in the laboratory, has practically closed the question of scarification so far as alfalfa is concerned.

GERMINATION OF FROSTED SEEDS

The germination of the immature seeds of sweetclover and of those discolored by frost has already been discussed (11). Immature seeds of alfalfa are relatively rare, but a small percentage of brown seed is very commonly present and is an important factor in the commercial value of the seed. The crop of 1925 contained an unusually large amount of seed discolored by frost. The germination of the brown seeds was found to be quite good if they were plump and of good weight, but it seemed desirable to determine whether such seeds would retain their viability. A series of 5 samples of alfalfa and 2 of sweetclover, all North Dakota grown, were assembled. All samples were carefully recleaned and approximately 20 g of each was separated by hand into brown and yellow, and stored in separate packages. The character of the samples is indicated in table 4.

TABLE 4.—*Quality of North Dakota grown alfalfa and sweetclover seed samples as affected by the presence of brown seeds discolored by frost*

Seeds and sample no.	Place grown in North Dakota	Percentage brown (by weight)	Weight of 200 seeds	
			Yellow	Brown
Alfalfa:			Grams	Grams
47581.....	Hastings.....	34.8	0.360	0.340
48248.....	Washburn.....	37.4	.420	.380
48770.....	Arnegard.....	41.2	.405	.360
48828.....	Washburn.....	46.2	.415	.380
49053.....	Lark.....	39.4	.415	.395
Sweetclover:				
47450.....	Christine.....	51.5	.390	.370
48024.....	Horace.....	31.8	.420	.385

The results of these tests were somewhat more difficult to interpret than those of the previous experiments because the condition of "hard" seeds is not so easily recognized in the brown condition. In general it may be said that during 6 years of storage the average actual germination of the yellow seeds in the alfalfa remained constant at about 57 percent, while the hard seeds decreased from 25 to 12

percent. In the brown seeds, germination decreased from 54 to 30 percent, while the hard seeds remained practically constant at about 13 percent. In the sweetclover, germination decreased from about 70 to 50 percent in both yellow and brown seed, and the hard seeds remained essentially constant at about 30 and 25 percent, respectively.

These results differ from those previously obtained by the writer and by other workers in indicating a longer retention of the hard-coated condition by frosted seeds. As previously suggested, it was felt that there was some question about the brown seeds recorded as hard at the end of the usual test. In 1935 the hard seeds remaining at the end of the test were carefully reconsidered, but without any material change in the results. All of the remaining hard seeds were then scarified by rubbing with emery cloth and replaced in the germination chamber. In two of the alfalfa samples (nos. 48248 and 48828) about two-thirds of the brown hard seeds softened without producing normal sprouts. In the other samples this result was less marked, though in all samples there were more dead brown seeds than yellow ones. The average additional percentage of dead seeds added to the original result by scarifying the hard seeds was 15 and 3 for the brown and yellow sweetclover and 7.5 and 1 for the alfalfa, respectively. It thus appears that in some of these hard brown seeds the vitality of the seed had been destroyed without rendering the coat permeable. Since no test of this sort had been made previously it is not known whether this occurred in the first instance or during storage.

FIELD GERMINATION OF CLOVERS

Of various field trials, two were quite extensive and of special interest since they included aged and injured seeds. In 1927, Wm. M. Jackson, at the Ellendale (N. Dak.) Normal and Industrial School, carried out a series of tests outlined by the writer. Plantings were made on May 6, May 26, June 22, and July 13. The first two were the most successful. In the latter two germination was poorer and weeds were more troublesome. A total of 45 carefully selected samples was used in the following groups: (1) Check—1 alfalfa, 3 sweetclover; (2) hard seeds—4 alfalfa, 3 sweetclover; (3) broken (chipped and cracked) producing broken sprouts—5 alfalfa, 4 sweetclover; (4) immature—5 alfalfa, 4 sweetclover; (5) frosted—3 alfalfa, 2 sweetclover; (6) old—4 alfalfa, 1 sweetclover.

For each planting 200 seeds were used. The old seeds were from the same samples shown in table 1, and therefore were 14 years old. The frosted seeds were from the samples listed in table 4, and were only 1 year old. The average weight of the seeds in the immature lots was 60 to 70 percent of that of normal seeds. The samples with broken seeds produced from 18 to 59 percent of broken sprouts in the blotter tests. While these are not counted as germinated, it was suspected that there were other sprouts which would not succeed in producing plants. Expressing the results in terms of percentage of plants in the field to germination in blotters the averages for all four plantings were:

- (1) Checks—alfalfa 64, sweetclover 51.
- (2) Hard seeds—alfalfa 123, sweetclover 48.
- (3) Broken seeds—alfalfa 42, sweetclover 24.
- (4) Immature—alfalfa 23, sweetclover 23.
- (5) Frosted—alfalfa, yellow 59, brown 38; sweetclover, yellow 53, brown 40.
- (6) Old—alfalfa 41, sweetclover 37.

The check sample of alfalfa showed about 15 percent of hard seed in the blotter test, the sweetclovers less than 10 percent. In series 2 about 65 percent of hard seeds were present in both alfalfa and sweetclover, and here the alfalfa produced more than twice as many plants as the sweetclover; that is, a large number of the hard seeds of the alfalfa produced plants and apparently none or few of the sweetclover. In the other lots, normal seeds produced about half as many plants as in the blotter test, the old and discolored seeds 10 to 15 percent less, and the immature and broken seeds about one-fourth as many. The marked difference between the alfalfa and sweetclover in the broken seeds is probably due to the difference in behavior of the hard seeds (35 percent present).

A second and similar trial was carried out at Fargo, N. Dak., in 1928 by W. A. Davidson. The same samples were used for the old and discolored seeds, but for the other lots new samples were employed similar to those used in the previous trial. Plantings were made on May 22 and July 9. The first gave better results. The soil was very dry at the time of planting, but rain fell on June 7 and the soil was in good condition at the time of the second planting. The results were similar to those at Ellendale and calculated on the same basis were:

- (1) Checks—alfalfa 48, sweetclover 62.
- (2) Hard seeds—alfalfa 146, sweetclover 61.
- (3) Broken seeds—alfalfa 22, sweetclover 32.
- (4) Immature—alfalfa 24, sweetclover 27.
- (5) Frosted—alfalfa, yellow 70, brown 40; sweetclover, yellow 63, brown 36.
- (6) Old—alfalfa 46, sweetclover 33.

A field planting was made May 8, 1920, of machine-scarified and of unscarified samples from identical lots of fresh seed. Calculated as in the preceding paragraph, the results were:

Alfalfa, 5 samples—unscarified, in field, 147; scarified, germinated between blotters, 198, in field 181.

Sweetclover, 3 samples—unscarified, in field, 82; scarified, germinated between blotters, 148, in field 104.

Red clover, 1 sample—unscarified, in field, 73; scarified, germinated between blotters, 102, in field 67.

In the 1920 planting, the seedlings were removed as they appeared and the experiment was carried on for only 1 month. In the other two plantings, the plants were left during the summer. On the whole, the results are in fair accord with those of other workers, although they do not indicate the germination of hard seeds later in the season as reported by Leggatt (3) and Whitcomb (13), nor their better germination at higher temperatures as reported by Lute (4). The influence of the variable factors of soil and weather are obviously very great. The writer wishes to stress particularly the variability in condition of samples. A classification into "scarified" and "unscarified" often is meaningless because of the variable effects of the scarifying process. This has been shown by the writer (11, p. 15) in a study of sweetclover.

LONGEVITY OF SOYBEANS

A collection of 23 samples of soybeans (*Soja max* (L.) Piper) tested for the Department of Agronomy of the North Dakota Experiment Station in 1924, was again tested in 1926 and 1928, and a few were tested in 1929-31. One hundred seeds were used for each test. The crop years of 1921, 1922, and 1923 and seven varieties were represented. The combined results are presented in table 5.

TABLE 5.—*Viability of soybeans of the crops of 1921-23 after storage in laboratory, 100 seeds being used per test*

Item	Results after storage for indicated number of years								
	1	2	3	4	5	6	7	8	9
Samples.....number..	13	4	17	4	17	8	8	4	3
Average germination.....percent..	92	91	83	84	67	65	51	47	30

Four of these samples gave 80 percent or more germination in 5 years, while only two had dropped below 50 percent. One sample of the Chestnut variety gave 90 percent the eighth year, but fell to 47 percent the tenth year (the only record for the tenth year). One sample of Manchuria gave 90 percent the seventh year but dropped to 66 percent the eighth year. The Wisconsin Black variety usually has shown considerable hard seed in fresh samples, but the two lots of it in this series did not hold their viability as well as some other varieties. In 1931, three of the best and three of the poorest samples were planted in the garden. The results are shown in table 6.

TABLE 6.—*Comparison of laboratory and field germination of old samples of soybeans of different varieties*

Variety	Age	Germination in laboratory	Germination in field
	Years	Percent	Percent
Chestnut.....	10	47	39
Manchu.....	9	33	5
Manchuria.....	8	66	44
Mandarin.....	9	21	22
Manchuria.....	--	14	4
Minsoy.....	8	16	10

The results of these tests are in fair agreement, considering the small number of trials, and are sufficient to show that the samples were all capable of producing plants in the field. It may be mentioned that some of them are still continuing (1935) to produce volunteer growth each year as a result of spontaneous reseeding.

GERMINATION OF BROKEN SEEDS IN FLAX

The broken seeds of flax (*Linum usitatissimum* L.) present a considerable problem, as may be seen from the fact that all samples examined at the seed laboratory from the crop years 1931 and 1932 contained an average of 4.5 percent by weight of pieces comprising not more than one-half of a seed. This includes samples of all kinds, cleaned and uncleaned. Occasionally the amount runs as high as 15 percent. The seeds may be broken in various ways, but reference is made especially to those that are broken transversely. In determinations of purity, according to the rules of the Association of Official Seed Analysts of North America (12), pieces of not more than one-half the size of a seed are placed in "inert matter", while pieces exceeding one-half are placed in "pure-seed." Although this procedure results in placing pieces from the larger end of the seeds

which contain no plumules with the seeds used for germination, it has been retained as the simplest working basis for seeds of most species.

From the 1930 crop, 11 cleaned samples were selected for germination experiments. All were of the Bison variety, grown in seven different counties, well distributed over North Dakota. Each sample was separated carefully into five divisions which gave an average composition in percentage by weight as follows: (1) Perfect seeds, 80.5; (2) slightly cracked, 6.8; (3) only small pieces broken off, 4.3; (4) plumule ends; more than one-half of a seed, 1.8; (5) plumule ends, less than one-half, 2.3. The separation into the five groups involved a considerable element of personal judgment, particularly in group 3, where minute pieces of seed coat may be broken from the plumule end in as much as 20 to 30 percent of the seeds. Such seeds were not used. The separations were made on 20 g by a single experienced worker after various comparative trials had been made by different workers and many pieces had been weighed. Seeds were tested in soil in the germinating chamber and a field planting was made about May 20. The soil in the field plot was firm and in good condition, and although the weather turned unusually warm a few days after the seeds were planted, germination and growth were good. The counts recorded in the field are of plants finally produced. The results of the tests are shown in table 7 and figure 2.³

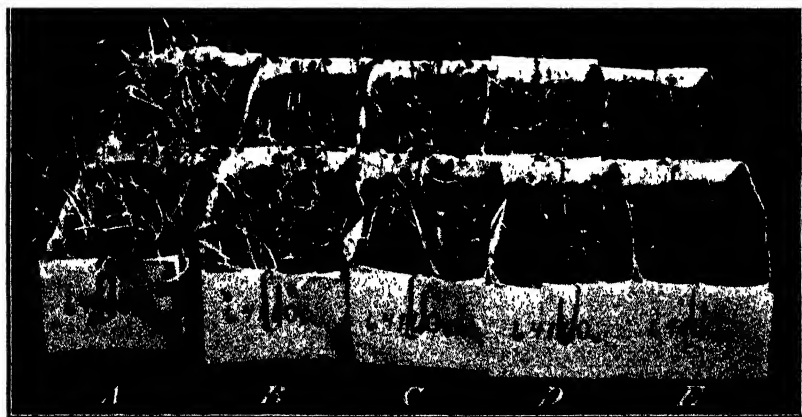


FIGURE 2.—Germination of normal and of broken seed of flax in soil in the germinating chamber, showing sample no. 64160, in front, and no. 63473, behind: A, Normal seed; B, slightly cracked seed; C, seed with only small pieces broken off; D, plumule ends, more than one-half of a seed; E, plumule ends, less than one-half of a seed.

It was somewhat of a surprise that even a few plants were produced by the small pieces. The variation in the samples in the field was striking, but it seemed to be mainly accidental as similar differences were not found in the soil tests in the chamber. No other sample gave such a high percentage of plants in the field from the normal seeds as no. 64160, yet all of the broken lots from this sample gave very poor results. The behavior of this sample in the chamber

³ Special credit is due Isabel Barrett for the laboratory work on this experiment as well as for work on other projects over several years. Charlotte and Anita Mary Blake did a considerable amount of the work on the earlier projects.

was quite similar to that of no. 63473; in fact it gave the highest number of sprouts from group 2.

TABLE 7.—Germination (percent) of perfect and of broken flaxseed in soil in the germination chamber and in the field

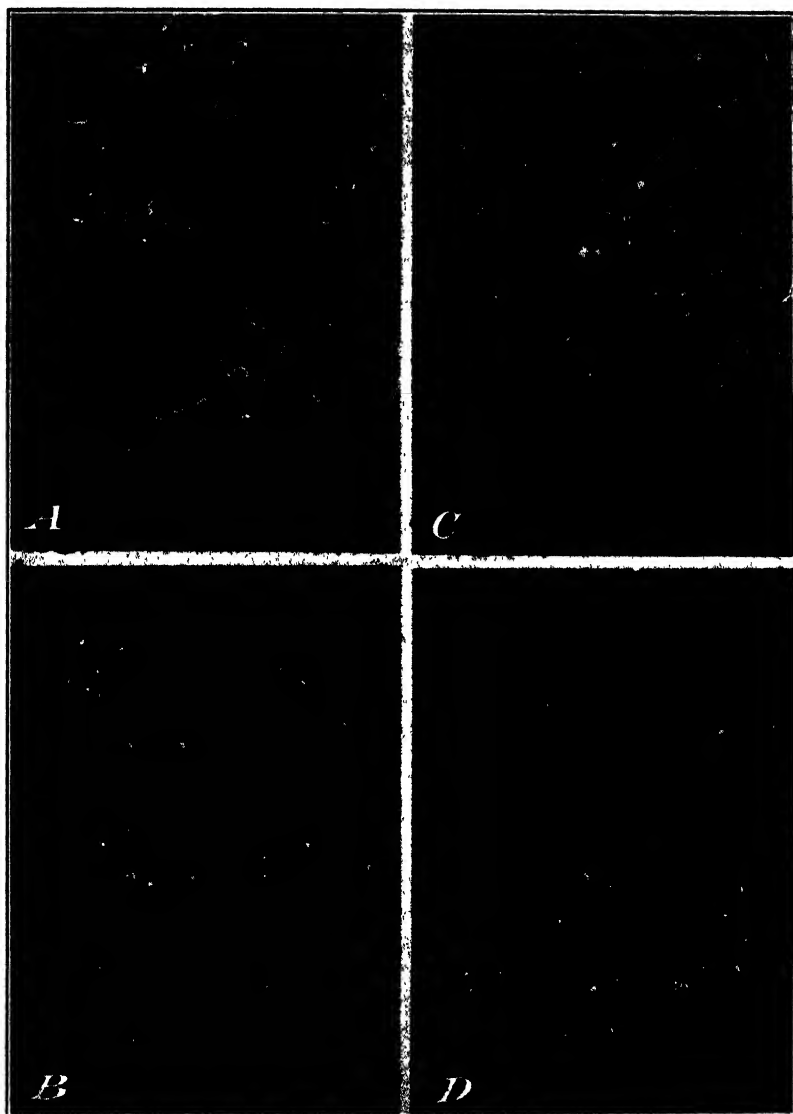
Sample no.	Perfect seed		Cracked seed		Slightly broken seed		More than half a seed		Less than half a seed	
	Chamber	Field	Chamber	Field	Chamber	Field	Chamber	Field	Chamber	Field
64160.....	97	90	86	12	73	7	51	0	9	0
63473.....	91	69	64	52	55	50	48	19	28	7
Average of 11 samples.....	89	63	73	30	57	20	38	8	13	3
Weak growths.....	2	-----	7	-----	11	-----	17	-----	15	-----

GERMINATION AND LONGEVITY OF HULLED TIMOTHY AND OTHER HULLED SEEDS¹

Several workers who have reported (1, 2) on the relative germination of hulled (naked caryopses) and unhulled (enclosed by lemma and palea) grains of timothy (*Phleum pratense* L.) found a considerably lower average germination for the hulled grains, but they did not carry the study further. McRostie (5) concluded (on the basis of one lot only?) that no serious deterioration should take place in 3 or even 4 years of storage. Newton and Ficht (6) found a rather rapid decline in germination of the hulled grains, but this was not reflected in the field because the rate of seeding was sufficiently heavy to provide enough plants. From their germination tests the one lot used appears to have been of representative character. The present writer reported (10) upon a survey of 77 samples received in the year 1923-24. From these, 10 samples which contained from 36 to 63 percent of hulled grains were selected and stored with the samples of clovers already discussed. These have been tested in May of each year, using each time 200 each of hulled and unhulled, and placing the separations from a given sample on the same blotter in the chamber. Only samples which showed a high initial germination of the hulled grains were used. The average initial germination was 95.2 for the hulled and 98.6 for the unhulled.

Figure 3 shows that the viability of the unhulled seeds remained essentially unimpaired for 5 years, after which it declined with increasing rapidity. The hulled grains lost viability steadily from

¹ In North Dakota and neighboring States the term "hulling" is in common use for the process of removing the covering (pod, pericarp) from the seeds of legume forage crops. Especially in sweetclover, the seeds are termed "unhulled" in their original condition and "hulled" after the pericarp has been removed. It should be noted that in this sense the term "hulled" refers to the result of a mechanical operation. Scientific writers have often described the grains of grasses as "hulled" when normally enclosed by the lemma and palea after threshing. "Hull-less" varieties are those which normally are freed from the lemma and palea in threshing. The naked condition in timothy and in the common varieties of millet is abnormal. Perhaps "degiumed" would be a better term for these, but it appears impossible to establish a simple and precise terminology for so many variants. The usage employed in the present paper is that commonly used commercially.



Germination of hulled and unhulled timothy seed after 8 years' storage: *A* and *C*, hulled, *B* and *D*, unhulled; *A* and *B*, sample no. 42260; *C* and *D*, sample no. 43869. No. 42260 gave the best results for hulled seed and no. 43869 the poorest.

the first, and in the better samples the results tended to approach those of the unhulled grains. In addition to these differences there were variations between individual samples, distinct breaks occurring in various years.

Personal judgment might seem to account for some of the apparent irregularities, but these usually concerned only certain samples, no general differences appearing in all of the samples in any one year. The sprouted grains from the test in 1932 of one of the best and one of the poorest samples are shown in plate 1. A soil test of no. 42260 made in the chamber on the same date gave 40 and 83 percent

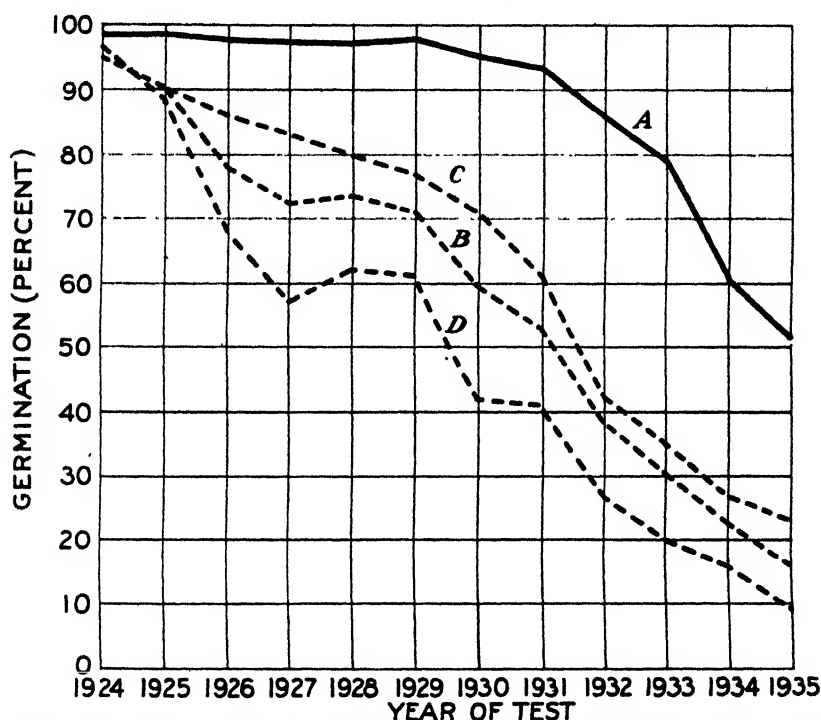


FIGURE 3.—Germination of hulled and unhulled timothy in storage. Average for unhulled (A) and hulled (B) in 10 samples; average of 6 best (C) and 4 poorest (D) of the hulled.

for the hulled and unhulled as compared with 61 and 92 for the blotter test and 89 and 98 for a soil test of a fresh check sample.

It was noted in 1934 that germination continued after the 8-day period, and when the seeds were left for 9 days more, an average additional 6 and 17 percent was recorded for the hulled and unhulled seeds, respectively. There were many grains producing shoots but no roots (therefore not counted as germinating), these averaging 5 and 6 percent, respectively, for the hulled and unhulled, but with the greatest variation in the latter. The unhulled seeds of sample no. 43869 retained their vitality best of all the samples, but the hulled seeds made one of the poorest showings. The information regarding this sample indicated that it was overripe and had stood in the shock, but was not damaged by rain.

It appears from these tests that while the hulled seeds usually germinate more poorly than the unhulled, in some samples they may be little inferior at first but lose vitality more rapidly than the unhulled. There may even be wide differences in rate of loss between the two kinds in one sample.

For several years comparative tests have been made whenever samples were available of the germination of the naked caryopses of grains which normally remain enclosed by the lemma and palea, particularly those of emmer (*Triticum dicoccum*) and quackgrass (*Agropyron repens*). The results are summarized in table 8.

TABLE 8.—Germination of various seed in hulled and unhulled condition

Kind of seed	Samples	Average content of hulled seed	Average germination	
			Hulled	Unhulled
	Number	Percent	Percent	Percent
Oats (<i>Avena sativa</i>).....	12	13	81	97
Emmer (<i>Triticum dicoccum</i>).....	1	(1)	18	74
Proso millets (<i>Panicum mitaceum</i>) ..	19	20	98	95
Foxtail millets (<i>Setaria italica</i>).....	9	22	51	94
Bromegrass (<i>Bromus inermis</i>).....	1	18	53	91
Reed canary grass (<i>Phalaris arundinacea</i>).....	1	10	72	96
	1	37	16	78
	1	100	5	-----
Quackgrass (<i>Agropyron repens</i>).....	1	100	31	-----
	1	70	24	86
	1	93.6	15	91
Yellow bristle grass (<i>Setaria puleascens</i>).....	4	100	24	-----
Green bristle grass (<i>Setaria viridis</i>).....	13	56	46	86
Buckwheat (<i>Fagopyrum esculentum</i>).....	1	3	98	98
Wild buckwheat (<i>Polygonum convolvulus</i>).....	6	-----	27	17

¹ Old and moldy.

In these tests it was found that the hulled seeds germinated more poorly, but with large variations, some samples showing no differences between the hulled and the unhulled. The hulled oats varied from 63 to 98 percent and with little relation to the amount of hulled grains present.

The millets are probably of most importance. A record has not been kept of the percentage of hulled grains in all samples, but in the proso millets it frequently reached 20. In the foxtail millets it was much lower. The bristle grasses were obtained from samples of alfalfa and sweetclover, chiefly from the 1927 crop which contained large quantities of these seeds. The lemma and palea envelop the caryopsis closely, but are frequently removed by the close hulling which the seed receives. The radicle of the embryo often is broken in the process. The yellow bristle grass, because of its larger size, is nearly always hulled and very often broken. An outstanding sample among the bristle grasses was one in which 93 percent of the grains were hulled and 87 percent of them germinated, the highest germination in the entire group. In another sample containing 50 percent hulled seed, 71 percent germinated, the second highest test, while of the unhulled seed 70 percent germinated, the lowest by 12 percent, for the group. Bristle grass germinated readily in all cases.

In quackgrass, the lemma and palea adhere tightly and the radicle projects distinctly so that it is very often broken a little. The fourth sample reported in table 8 was secured from a dealer who stated

that it came from new-crop sweetclover which had just been hulled and scarified. The first test was made April 1. On May 1 and June 1, the hulled quackgrass gave the same germination, but on December 1 it gave only 4 percent, the unhulled 91 as before.

Wild buckwheat is a common impurity, and in alfalfa or other small seeds it becomes considerably broken. The embryo lies close to the surface, on one angle, and so is likely to be injured in hulling, which in this case means removal of the pericarp. The germination tests on this seed were unsatisfactory, as the normal seeds failed to respond in 3 cases out of 4. Nor did they respond to any extent to chipping, testing in soil at 18° C., or on blotters at 14°. The ordinary buckwheat (*Fagopyrum*) is not at all comparable to wild buckwheat, for its embryo lies in the center of the seed.

LONGEVITY OF PERENNIAL SOWTHISTLE SEEDS

The achenes of *Sonchus arvensis* have germinated readily with the usual 20°–30° C. alternation as previously reported (9), and quite uniformly have produced about 95 percent. Many tests have been made, but no regular annual ones. Fresh seed has been collected nearly every year and as tested May 1, 1935, these lots showed a poor growth for the 1929 crop and none for the crops of 1922 to 1928.

SUMMARY

Annual germination tests were made upon various seeds stored in the laboratory up to 20 years. Under these conditions the viability of good alfalfa and sweetclover seed declined steadily from about 95 to about 60 percent in 20 years. Red clover dropped to about 10 percent.

Hard seeds in alfalfa became permeable to a large extent during the spring months, and while few hard seeds remained after 1 year, from 1 to 4 percent were present even after 20 years. In red clover there was a slower decrease in hard seeds, and in sweetclover very little, two samples retaining at 20 years one-half and four-fifths, respectively, of their original hard-seed content. Some samples which originally contained few hard seeds retained their vitality as well as others. Seeds of alfalfa and sweetclover touched with frost when maturing showed greater retention of hard seeds than did normal lots, but in some cases these did not produce normal sprouts when scarified.

In field plantings of samples containing a high percentage of hard seeds, alfalfa produced from one-fourth to one-half more plants than was indicated by blotter test, while alfalfa which did not contain a large amount of hard seeds and sweetclover both with and without hard seeds, produced only about one-half as many plants as indicated by the blotter test.

Twenty-year-old seeds of alfalfa and sweetclover, and also fresh seeds discolored by frost but of nearly normal weight, gave results in the field only slightly lower in proportion to their blotter test than the normal seeds. Broken and quite immature seeds gave only about one-half as good results.

Soybeans declined steadily but retained an average viability of 30 percent at 9 years.

Broken seeds of flax gave poor germination, but even pieces of less than one-half of a seed gave a maximum of 12 (average 3) percent of plants in the field.

Normal grains of timothy retained normal viability 5 or 6 years, then declined rapidly to 60 percent in 10 years. Hulled (deglumed) grains from the same samples declined steadily to about 20 percent in 10 years, but the better lots decreased to only 70 percent in 6 years.

Millet, especially the proso types, often had a considerable proportion of grains without glumes, and these germinated poorly. Grains of green bristle grass, found in samples of clover and deglumed through clover hulling, were similarly injured. Grains of quackgrass were commonly broken in the same process but gave as high as 30 percent germination.

Seeds of perennial sowthistle retained their viability for 5 years only. Wide differences were observed in the behavior of apparently similar samples, and the causes and extent of variations in such tests is discussed.

LITERATURE CITED

- (1) GOSS, W. L.
1916. GERMINATION OF HULLED AND UNHULLED TIMOTHY SEEDS AS THEY OCCURRED IN SAMPLES RECEIVED AT THE SEED LABORATORY. Assoc. Off. Seed Anal. North America Proc. (1915) 8: 21.
- (2) HARLING, MRS. E. P.
1923. HULLED AND UNHULLED TIMOTHY. Seed World 13 (6): 24.
- (3) LEGGATT, C. W.
1927. THE AGRICULTURAL VALUE OF HARD SEEDS OF ALFALFA AND SWEET CLOVER UNDER ALBERTA CONDITIONS. Sci. Agr. 8: 243-266, illus.
- (4) LUTE, A. M.
1928. IMPERMEABLE SEED OF ALFALFA. Colo. Agr. Expt. Sta. Bull. 326, 36 pp., illus.
- (5) McROSTIE, G. P.
1923. HULLED VERSUS UNHULLED TIMOTHY SEED. . . . Seed World 13 (9): 26.
- (6) NEWTON R., and FICHT, J.
1926. EXPERIMENTS WITH TIMOTHY. Alberta Univ., Col. Agr. Research Bull. 3, 87 pp., illus.
- (7) SIFTON, H. B.
1920. LONGEVITY OF THE SEEDS OF CEREALS, CLOVERS, AND TIMOTHY. Amer. Jour. Bot. 7: 243-251.
- (8) STEVENS, O. A.
1918. VARIATIONS IN SEED TESTS RESULTING FROM ERRORS IN SAMPLING. Jour. Amer. Soc. Agron. 10: 1-19, illus.
- (9) ———
1924. PERENNIAL SOW THISTLE: GROWTH AND REPRODUCTION. N. Dak. Agr. Expt. Sta. Bull. 181, 44 pp., illus.
- (10) ———
1925. GERMINATION OF HULLED TIMOTHY SEED. Seed World 17 (4): 14.
- (11) ——— and LONG, H. D.
1926. SWEET CLOVER SEED STUDIES. N. Dak. Agr. Expt. Sta. Bull. 197, 20 pp., illus.
- (12) UNITED STATES DEPARTMENT OF AGRICULTURE.
1927. RULES FOR SEED TESTING. U. S. Dept. Agr. Circ. 406, 13 pp. (Revised, 1928).
- (13) WHITCOMB, W. O.
1931. HARD SEEDS IN LEGUMES: INTERPRETATION OF THEIR VALUE AND METHODS OF TREATMENT. Mont. Agr. Expt. Sta. Bull. 248, 63 pp., illus.

THE NUTRITIVE VALUE OF SKIM-MILK POWDERS, WITH SPECIAL REFERENCE TO THE SENSITIVITY OF MILK PROTEINS TO HEAT¹

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INTRODUCTION

In the commercial dehydration of raw liquid skim milk the immediate practical objective is the production of a dry powder that may be conveniently stored and readily transported. The agency of dehydration is heat. In the merchandising of these powders emphasis is placed upon their high nutritive value, particularly as sources of protein, of calcium, and of vitamin G. It is commonly, if tacitly, assumed that they are equal in nutritive value to the solids of raw liquid skim milk. However, there is more than a remote possibility that the heat treatment to which they have been subjected has lowered their nutritive value, although little definite evidence to this effect has been found in the literature.

In the drying of liquid skim milk, there are times when the resulting products are scorched. This scorching is caused in different ways, and under careful management it may be prevented. The point of interest here is that these scorched products are offered for sale through the various merchandising channels. In such cases there is ocular evidence that processing has changed the milk solids, but to what extent the nutritive value has deteriorated there is again no available evidence.

While many types of equipment are used in the manufacture of dry skim-milk powders, only two distinct processes of dehydration are in general use, one the roller process, and the other the spray process. Powders made by the roller process are largely merchandised as animal feed, while the powders sold for human consumption are largely prepared by the spray process. Whether the powders produced by the two processes are of equivalent nutritive value is not definitely known.

Of the 288,000,000 pounds of dry skim-milk powder produced in the United States in 1933, about 60 percent went into human consumption through such media as bakery goods, ice cream, confectioneries, and breakfast cereals. The remaining 40 percent was sold as animal feed. The former channels of consumption obviously represent the most particular and fastidious of sales distribution, in which a scorched product is frowned upon because of its off color and taste. The animal-feed buyer is not opposed to the scorched product because of color or taste, but he does, and should, view it with some doubt on the score of its nutritive value.

¹ Received for publication July 11, 1935; issued February 1936. The experimental data reported in this article have been taken in part from the thesis of B. W. Fairbanks submitted in 1935 to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of doctor of philosophy in animal husbandry, and in part from an experiment station project. The investigation carried on by the senior writer was made possible by the donation of funds and samples of skim-milk powders to the University of Illinois by the American Dry Milk Institute, Inc.

The investigation reported in the following pages was planned to solve these problems, in part at least, but mainly as they relate to the changes in the nutritive value of milk solids induced by the heat processes employed in the commercial manufacture of skim-milk powders.

REVIEW OF LITERATURE

Nevens and Shaw (13)² showed that the digestibility of the proteins of whole-milk powder was appreciably less than that of the proteins of liquid whole milk. They did not observe any significant differences in the digestibility of the proteins of whole-milk powders produced by the roller and the spray processes, although Miyawaki, Kanazawa, and Kanda (11) claim, on the basis of *in vitro* digestion experiments, that the digestibility of the proteins of roller-process powder is somewhat less than that of spray-process powder.

Chick (2) and Goldblatt and Moritz (5) compared the growth-promoting values of unheated and heated casein, obtaining no evidence of a deleterious effect of heat. In these tests the casein was heated for 36 to 72 hours at 110° to 130° C. As high-protein diets were used in both investigations, a possible slight inferiority of the heated casein may well have been masked. The more rapid resumption of growth in rats following depletion of vitamin A when unheated casein was used than when casein heated at 105° C. for 7 days was used, noted by Coward, Key, Morgan, and Cambden (3), may well have been a result of heat upon the casein itself rather than upon any vitamin A originally present in it. Morgan (12) obtained consistently lower biological values for toasted (150° for 30 minutes) than for raw casein, and in later work with Greaves (6) an attempt was made to trace the heat (140° for 30 minutes) deterioration to the destruction of certain of the amino acid components of casein; *i. e.*, lysine, histidine, tyrosine, cystine, and tryptophane. From growth experiments they concluded that the nutritive value of both heated and unheated casein was improved by a supplement of cystine and that that of the heated casein (but not of the unheated casein) was improved by supplements of either lysine or of histidine; in nitrogen-balance experiments they found that the biological value of unheated as well as of heated casein was increased by a cystine supplement, while that of the heated casein was improved also by a supplement of lysine. Such results, indicating a supplementation of proteins simultaneously by more than one amino acid indispensable to life, are contrary to prevailing conceptions of the relation between the composition of proteins and their value in promoting protein synthesis in animals. Block, Jones, and Gersdorff (1) found that the lysine yielded by the acid hydrolysis of casein was not lowered by previous treatment of the casein with dilute sodium hydroxide or by dry heat (150° for 65 minutes).

The above-cited experiments on heated versus unheated casein are of no definite value in predicting the effect on the nutritive value of milk solids of dehydrating by the prevailing commercial processes, mainly because the time of exposure to heat is so much shorter in the latter case. Thus, in the roller process, the milk film is in contact with the drying roll for only about 4 seconds, and for only a fraction of this time is it exposed to the maximum roller temperature, estimated at

² Reference is made by number (italic) to Literature Cited, p. 1120.

134° C.³ In the spray process the raw milk is preheated at 60° to 63°, concentrated in a vacuum pan, and sprayed into a current of air at temperatures ranging from 93° to 149°.

OBJECT, MATERIALS, AND METHODS

The object of the experiment was to compare the digestibilities and the biological values of the proteins of a series of skim-milk powders prepared by different methods and processes with each other and with those of a sample of raw skim milk which had not been subjected to any heat treatment whatever. Albino rats were used as subjects. Somewhat incidentally, the net-energy values of the two roller-process powders subjected to the severest and the mildest heat treatment were compared. A study was also made of the amino acids limiting the nutritive values of the proteins of the roller-process powders, for the purpose of determining the location of the heat injury.

Six samples of dry skim-milk powders were prepared in the Dairy-men's League plant at Massena, N. Y., from the same supply of milk. Four of the samples were made upon twin-cylinder atmospheric rolls and the remaining two were prepared by the continuous spray process. These six samples were compared to raw liquid skim milk.

Raw liquid skim milk.—This sample was obtained from the Division of Dairy Manufactures of the University of Illinois and was drawn before any heat was applied. While this sample of skim milk was not taken from the liquid skim milk from which the powders were prepared, the discrepancy is not serious, as the proteins of skim milk are comparatively constant in amount and proportions.

Low-temperature powder, roller process (r. p.).—The steam gage at the rolls registered 50-pound pressure, and the film of milk delivered to the roll was thinner than in common practice. This powder was exposed to a temperature less than that commonly employed in commercial drying.

Choice commercial powder, roller process (r. p.).—Ordinary commercial plant procedures were employed, including 87 pounds of steam pressure and a milk film of ordinary thickness. This sample was a high-quality product, and would receive the highest grade adopted by the standards committee of the American Dry Milk Institute.

Slightly scorched powder, roller process (r. p.).—The steam pressure was increased to 90 pounds and a thin film of milk was delivered to the rolls. This sample was representative of the scorched milk powder frequently encountered in the trade.

Scorched powder, roller process (r. p.).—The knives were lifted intermittently from the rolls, so that the milk solids made more than one revolution of the rolls. The product was representative of the extreme scorching occasionally found in market samples.

Not preheated powder, spray process (s. p.).—The usual preheating process was omitted. Such a powder is not frequently encountered in the trade.

Preheated powder, spray process (s. p.).—The usual preheating process which is considered necessary for the preparation of a high quality good-baking spray-process powder was included.

³ No estimate of the maximum temperature to which the milk film is exposed. High degree of accuracy not be generally applicable under all conditions.

All samples were shipped to Urbana in tin containers with tight-fitting lids, except the choice commercial powder, which was shipped in a well-made barrel lined with paper. On arrival the latter sample was transferred to 50-pound lard pails. All samples were stored in a dry place.

For purposes of precise description, the chemical composition, solubility, and color analysis of the skim-milk powders are given in table 1.

TABLE 1.—Chemical composition, solubility, and color analysis of the experimental dry skim-milk powders

Sample of powder	Chemical composition					Solubility		Color analysis		
	Dry substance	Nitrogen	Crude protein (N×6.25)	Fat	Gross energy per gram	Dry substance in solution	Nitrogen in solution	Brightness	Dominant wave length	Purity
	Per-cent	Per-cent	Per-cent	Per-cent	Calorien	Per-cent	Per-cent	Per-cent	m μ	
Low-temperature, r. p.	96.12	5.41	33.81	0.91	4.06	71.3	34.6	84.5	600	1
Choice commercial, r. p.	98.06	5.50	34.38	.99	4.02	67.7	32.4	84.6	560	23
Slightly scorched, r. p.	99.87	5.41	33.81	1.07	4.02	62.4	27.9	77.1	580	17
Scorched, r. p.	98.90	5.55	34.69	.90	4.25	64.6	24.9	62.0	587	18
Not preheated, s. p.	98.32	5.40	33.75	.69	4.13	95.1	92.3	95.5	590	1
Preheated, s. p.	98.40	5.50	34.38	.62	4.12	92.6	91.6	94.5	580	4

The method for determining solubility was adapted from the method of Wright (17). Twenty grams of skim-milk powder were transferred to a beaker, to which was added 200 cc of nitrogen-free water at 20° C. The beaker was placed in a water bath of 20°, and the contents stirred mechanically for exactly 30 minutes. Fifty cubic centimeters of the mixture were transferred to each of two centrifuge tubes and whirled at 1,850 revolutions per minute for exactly 15 minutes. Three 10-cc samples of the supernatant fluid were removed from one centrifuge tube for moisture determination, while from the second centrifuge tube three 10-cc samples were taken for the determination of nitrogen.

The colors were analyzed with a Keuffel and Esser color analyzer or spectrophotometer. For these analyses the samples were ground in a mortar until all material passed through a 100-mesh sieve. The three characteristics of color—brightness, dominant wave length, and purity—are expressed numerically. As most of these powders were white or very nearly so, it is believed that the figures for brightness are the most significant.

The data of table 1 show that, with a single exception for solids and none for nitrogen, the solubility of the roller-process powders decreased as the severity of the heat treatment increased. The spray-process powders were much more soluble than the roller-process powders, and of the two former the preheated was the less soluble. Also the spray-process powders possessed a greater brightness rating than the roller-process powders, while among the latter, brightness decreased as the intensity of heat processing increased.

In many of the experiments the paired-feeding method (9) was used, while for the determination of the biological values of protein the nitrogen-balance method developed in this laboratory (7, 10) was followed.

EXPERIMENTAL RESULTS

THE DIGESTIBILITY OF ENERGY

The apparent digestibility of energy was determined upon two of the roller-process powders, the low-temperature powder and the scorched powder, representing the two extremes of heat treatment. This determination was made upon eight pairs of rats which were being used in a feeding experiment designed to compare the net-energy values of these powders. The results of the net-energy test are presented below. The rations compared contained about 63 percent of the respective powders. The collection periods were of 7 days duration and a feces marker was used.

The average coefficients of apparent digestibility of the energy of these two diets were 90.5 for the low-temperature powder and 89.2 for the scorched powder. In all pairs a higher coefficient was obtained with the low-temperature powder. Such a consistent result would be produced by chance only once in 128 trials, so that its significance is established without further statistical analysis. It may be concluded that the extreme heat employed in preparing the scorched powder depressed the digestibility of its energy in the animal body. Although the average depression for the ration as a whole was 1.375 percent, that of the powder itself, making up only 62.3 percent of the ration, must have been $1.375 \div 0.623 = 2.21$ percent.

THE DIGESTIBILITY OF PROTEIN

The digestibility of the protein in raw liquid skim milk and in the various skim-milk powders was determined from the nitrogen metabolism data secured for the purpose of computing the biological values of these protein mixtures. In these metabolism trials the various samples were compared in turn with the low-temperature (r. p.) powder, the same rats being used in each comparison in groups of 5, 8, or 10. The average coefficients of true digestibility (including due allowance for the metabolic products in the feces) are given in table 2.

TABLE 2.—Average coefficients of true digestibility of protein for the various experimental skim-milk samples

Rats (number)	Raw liquid skim milk	Roller-process powders				Spray-process powders	
		Low temperature	Choice commercial	Slightly scorched	Scorched	Not preheated	Preheated
8	94.8	92.7	93.4	88.8	81.4	92.0	95.3
5		90.6					
5		91.7				92.0	
5		92.1					

¹ Average of 10 determinations, 2 on each rat.

If digestion coefficients obtained for any two samples on the same rat are considered as paired observations, then Student's (15) method for the statistical analysis of small groups of such data may be applied. Such an analysis of the individual data shows that the proteins of liquid skim milk are significantly more digestible than the proteins of

the low temperature (r. p.) powder, and that the latter proteins are significantly better digested than those of the slightly scorched (r. p.) and scorched (r. p.) powders. All other differences are statistically insignificant.

These results are only in partial agreement with those of Nevens and Shaw (13), who were able to demonstrate a significantly greater apparent digestibility of the proteins of fresh whole milk than of the proteins of whole-milk powder prepared by either the spray process or the roller process. In these studies the sole diet of the experimental rats consisted of the milk products under test, while in the investigations reported in this article the experimental diets contained less than 25 percent of milk solids. The former dietaries would be more favorable to the detection of differences in the digestibility of milk products than would the latter.

THE NET-ENERGY VALUE

The two skim-milk powders representing the two extremes in heat treatment; i. e., the low-temperature and the scorched roller-process powders, were compared with reference to their availability as sources of energy in animal nutrition. They were incorporated into rations containing other sources of protein, minerals, and vitamins in presumably adequate amounts, so that the milk solids need supply only energy-yielding nutrients. The composition of the rations is given in table 3. The water was added to diet 2 in order to equalize the moisture content of the two powders (table 1).

TABLE 3.—Composition of the diets used in the comparison of the net-energy values of low-temperature (r. p.) skim-milk powder and scorched (r. p.) powder

Constituent	Diet 1	Diet 2	Constituent	Diet 1	Diet 2
	Percent	Percent		Percent	Percent
Low-temperature (r. p.) powder.....	63.50	62.34	Dried yeast ¹	5.00	5.00
Scorched (r. p.) powder.....			Cod-liver oil.....	2.00	2.00
Casein.....	25.00	25.00	Water.....		1.16
Modified Osborne and Mendel salts ¹	4.50	4.50			

¹ See Wasson (16).

² Obtained from the Northwestern Yeast Co.

The rations were compared with respect to growth-promoting power by means of the paired feeding method, using eight pairs of rats. The growth results are assembled in table 4.

TABLE 4.—Comparison of the net-energy value of low-temperature powder (r. p.), diet 1, and scorched powder (r. p.), diet 2, as determined by the paired-feeding method during a feeding period of 56 days

Pair no. and powder used in diet	Total food	Initial weight	Final weight	Gain	Pair no. and powder used in diet	Total food	Initial weight	Final weight	Gain
Pair 1, males:					Pair 5, males:				
Low-temperature powder.....	Grams 527	Grams 49	Grams 155	Grams 106	Low-temperature powder.....	Grams 446	Grams 40	Grams 131	Grams 91
Scorched powder.....	527	51	145	94	Scorched powder.....	446	40	113	73
Pair 2, males:					Pair 6, females:				
Low-temperature powder.....	557	50	160	110	Low-temperature powder.....	469	46	122	76
Scorched powder.....	557	47	157	110	Scorched powder.....	469	46	126	80
Pair 3, females:					Pair 7, females:				
Low-temperature powder.....	527	48	134	86	Low-temperature powder.....	423	46	109	63
Scorched powder.....	527	50	144	94	Scorched powder.....	423	53	109	56
Pair 4, females:					Pair 8, males:				
Low-temperature powder.....	479	41	124	83	Low-temperature powder.....	499	52	143	91
Scorched powder.....	479	40	123	83	Scorched powder.....	499	51	151	100

After the feeding period was well under way, it appeared that both diets were deficient in vitamin B. In order to maintain appetite, each rat was given daily by mouth from 3 to 6 drops of tikitiki extract, pair mates being treated exactly alike.

The gains in weight of pair mates reveal no superiority of diet 1 over diet 2. In 3 pairs the rat on diet 1 gained the faster, in 3 pairs the reverse was true, while in 2 pairs the gains were equal.

Since it was possible that a difference in net-energy value between diets 1 and 2 produced a difference in the energy balance rather than in the rate of gain of the rats, the carcasses of four pairs, nos. 1, 3, 5, and 8, were analyzed for gross-energy content, using the Parr oxygen bomb calorimeter. Their contents of nitrogen were also determined. The results are summarized in table 5.

TABLE 5.—*Nitrogen and energy contents of the carcasses of 4 pairs of rats reared on diets 1 and 2*¹

Pair no.	Dry skim-milk powder in diet	Empty body weight	Nitrogen content		Energy content	
					Per gram	Total
		Grams	Percent	Grams	Calories	Calories
1.....	(Low temperature.....	150	2.86	4.29	1.32	198
	(Scorched.....	139	2.77	3.85	1.32	183
3.....	(Low temperature.....	129	2.94	3.79	1.32	170
	(Scorched.....	138	2.44	3.37	1.32	182
5.....	(Low temperature.....	127	2.79	3.54	1.33	169
	(Scorched.....	108	2.86	3.09	1.32	143
8.....	(Low temperature.....	138	3.04	4.20	1.31	181
	(Scorched.....	145	2.96	4.29	1.31	190

¹ Diet 1 contained the low temperature and diet 2 the scorched powder.

The energy content per gram of empty carcass was remarkably constant for all rats examined, and the total energy content of pair mates showed no consistent differences induced by the two diets. In 2 pairs the rat subsisting on diet 1 stored the greater amount of energy in its body, while in the remaining 2 pairs the reverse was true.

The nitrogen content of the carcass of the rat receiving the low-temperature powder (diet 1) was higher, both on the percentage and the absolute basis, in 2 of the 4 pairs than that of the rat receiving the scorched powder, but these differences are not sufficiently large or consistent or numerous to indicate with any great degree of certainty, according to statistical analysis, that they were the result of the difference in diet consumed rather than the result of chance.

The digestibility coefficients above discussed indicate clearly that the energy of the scorched skim-milk powder was somewhat less digestible than that of the low-temperature powder. The failure to demonstrate a difference in the net-energy values of the two powders by the growth experiment may be the result of (1) a greater degree of activity of the rats on the diet containing the low-temperature powder, (2) a greater specific dynamic effect of this diet or (3) a biological error in the growth experiment sufficient to obscure the effect of the greater digestibility of the low-temperature powder.

In all probability any difference in net-energy value between the low-temperature skim-milk powder and the scorched powder is inconsiderable. It may be concluded further that powders subjected to

heat insufficient in intensity to produce more than a barely perceptible scorching are for all practical purposes equally valuable as sources of energy in the animal body.

BIOLOGICAL VALUE OF THE PROTEINS

The general plan of experimental procedure in determining the biological value of the proteins was that developed by Mitchell (7, 10), except that in these metabolism studies feces markers⁴ were employed. The compositions of the various diets are presented in table 6. In order to improve the consumption of the experimental diets, Harris yeast vitamin powder was fed. This vitamin concentrate was fed in some instances as part of the diet, in others as part of the diet supplemented by additional amounts weighed into individual feed dishes, and later by feeding all of it as an addition to the mixed diet, which accounts for the presence of more than 1 percentage of this constituent in a given diet. As the amount of vitamin powder was changed in the diet, a corresponding and equal change was made in the amount of starch.

TABLE 6.—Composition of the diets used in studying the relative biological values of the proteins of raw liquid skim milk and the various dry skim-milk powders

Constituents	Stand- ardiz- ing diet	Raw liquid skim milk	Low- tem- pera- ture powder (r. p.)	Choice com- mercial powder (r. p.)	Slightly scorched powder (r. p.)	Scorched powder (r. p.)	Not pre- heated powder (s. p.)	Pre- heated powder (s. p.)
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Dried fat-free whole egg.....	{ 5.47 or 5.35 }							
Raw liquid skim milk.....		22.55						
Low-temperature powder (r. p.).....			23.66					
Choice commercial powder (r. p.).....				23.27				
Slightly scorched powder (r. p.).....					23.66			
Scorched powder (r. p.).....						23.06		
Not preheated powder (s. p.).....							23.70	
Preheated powder (s. p.).....								23.27
Modified Osborne-Mendel salts.....	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Filtered butterfat.....	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Cod-liver oil.....	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Sucrose.....	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
NaCl.....	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Harris yeast vitamin powder.....	{ .5 or .0 }		{ .3 or .5 or .0 }	.5	.5	.5		
Starch.....	{ 66.53 or 67.15 }	49.95	{ 48.54 or 48.34 or 48.84 }	48.73	48.34	48.94	48.80	49.20
Pigment.....	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

¹ Dry substance.

The calculations of the biological values of the proteins of the various milk samples was made from the nitrogen metabolism data according to the usual procedure followed in this laboratory (10); hence it seems unnecessary to give them in detail here. The biological

⁴ On the first day of each experimental period the diet fed contained 2 percent of ferric oxide, which marked the feces red. On the remaining days of the experimental period, in most cases of 7 days' duration, an equal percentage of barium sulphate was included in the diet. This produced a progressively lighter color in the feces. On the first day following an experimental period, or the first day of a transition period, which was always 4 days in length, the diet contained 2 percent of chromic oxide to mark the feces green.

value is the percentage of the absorbed nitrogen that is retained for both maintenance and growth under the standard conditions adopted for the determination. The estimate of the absorbed nitrogen makes due allowance for the metabolic nitrogen excreted in the feces, the ratio between metabolic fecal nitrogen and dry-matter intake being measured in the standardizing periods. The estimate of the total storage of nitrogen in the body makes due allowance for the endogenous nitrogen excreted in the urine, the ratio between endogenous nitrogen and body weight being also determined in the standardizing periods.

The various milk samples were compared in turn with respect to the biological value of their protein components with the low-temperature (r. p.) powder, groups of 5, 8, or 10 rats being used in each comparison. The average biological values for each group of rats and for each milk sample are assembled in table 7.

TABLE 7.—Average biological values of protein for the various experimental skim-milk samples

Rats (number)	Raw liquid skim milk	Roller-process powders				Spray-process powders	
		Low temperature	Choice commercial	Slightly scorched	Scorched	Not preheated	Preheated
8.....	89.8	89.1					82.3
5.....		89.6	81.6			87.2	
5.....		89.3		68.0		88.6	
5.....		87.9			69.8		

† Average of 10 determinations, 2 on each rat.

The average biological value of 89.8 for the proteins of liquid skim milk may be compared with the average of 95 obtained by Shiftan (14) with two pigs fed a ration containing about 12 percent of protein. The average of 82.3 for preheated skim-milk powder (s. p.) is very close to the value of 85 obtained in this laboratory in two investigations (8, 10) with milk powders of this description, and also with the value of 86 reported by Boas Fixsen and Jackson (2), relating to a whole-milk powder manufactured by the roller process.

The biological values (single or average) obtained for different samples on the same rats have been considered as paired observations, and the differences between them have been subjected to statistical analysis according to the method of Student (15). The statistical results are summarized in table 8. From these analyses it may be concluded that the nutritive value of the digestible proteins of fresh skim milk is not depressed by drying by the roller process at the lowest feasible temperature, nor by drying by the spray process provided the preheating is dispensed with. However, if drying is accomplished by the prevailing commercial processes, yielding products represented by the choice commercial (r. p.) sample and the preheated (s. p.) sample, a definite lowering of nutritive value occurs, equal to about 8 percent. If drying by the roller process is so poorly controlled that even slight scorching occurs, then a much greater decrease in nutritive value is effected, amounting to more than 20 percent.

TABLE 8.—Analysis according to Student's method of the differences in biological values between the proteins of dry skim-milk powders and of liquid skim milk

Statistical item	Raw liquid skim milk s. low-temperature powder (r. p.)	Low-temperature powder (r. p.) v. not preheated powder (s. p.)	Raw liquid skim milk s. preheated powder (s. p.)	Low-temperature powder (r. p.) v. preheated powder (s. p.)	Low-temperature powder (r. p.) v. choice commercial powder (r. p.)	Low-temperature powder (r. p.) v. slightly scorched powder (r. p.)	Low-temperature powder (r. p.) v. scorched powder (r. p.)
Mean of differences, <i>M</i>	0.625	1.7	7.5	6.875	7.8	21.6	18.2
Standard deviation of differences, <i>s</i>	4.22	4.24	3.54	3.14	1.6	2.32	3.25
Probability, <i>P</i>35	.13	.0004	.0003	<.0019	<.0019	.0019

TOTAL EFFECT OF DRYING UPON THE NUTRITIVE VALUE OF SKIM-MILK PROTEINS

The relative protein values of the various milk samples tested, which take account of differences in digestibility of protein as well as differences in biological value, have been computed and the results are presented in table 9. These values, listed in the last column of the table, are expressed in percentages, the value of raw liquid skim milk being taken as 100. From these values it appears that skim milk may be dried with a loss in protein value of only 5 percent or less, but that by the ordinary processes of commercial drying losses of 9 to 11 percent occur, and if there is perceptible scorching through careless management the loss may be 30 percent.

TABLE 9.—Relative protein values of the different milk samples tested ¹

Sample	Average true digestibility of protein	Average biological value	Relative protein value	Sample	Average true digestibility of protein	Average biological value	Relative protein value
Raw liquid skim milk.....	Percent 95	Percent 90	Percent 100	Spray-process powders:	Percent	Percent	Percent
Roller-process powders:				Not preheated.....	92	88	95
Low temperature.....	91	89	95	Preheated.....	95	82	91
Choice commercial.....	93	82	89				
Slightly scorched.....	89	68	71				
Scorched.....	81	70	66				

¹ Expressed as percentages with the relative protein value for raw liquid skim milk taken as 100.

Thus, the data obtained demonstrate a point of tremendous practical importance, namely, that the proteins of milk are very sensitive to heat with respect to their value in nutrition, and that considerable losses in protein efficiency may be incurred unless the time of exposure to heat and the intensity of the heat are carefully controlled. In this respect the requirements for the highest engineering efficiency may conflict with the requirements for the highest quality of product, and the dry-milk industry must decide which requirements are paramount.

SEAT OF INJURY TO THE PROTEINS OF MILK PRODUCED BY DRYING IN TERMS OF THE CONSTITUENT AMINO ACIDS

Having established the existence of definite deterioration of skim-milk proteins during commercial drying processes, as well as the

extent of such deterioration, the next step in the investigation was to discover, if possible, which of the constituent amino acids was involved in the successive decreases in nutritive value as the severity of the drying process was intensified. Such information would be afforded by a study of the amino acids limiting the nutritive values of the various samples of skim-milk powders.

In this study the paired-feeding method was used to compare the growth-promoting powers of rations containing the various milk powders as the sole source of protein and the same rations with small supplements (0.3 percent) of selected amino acids. The rations all contained enough of the milk powders (23 to 27 percent) to supply approximately 9 percent of protein, 4.5 or 5.0 percent of the Wesson (16) modification of the Osborne and Mendel salt mixture, 1 percent of NaCl, 10 or 12 percent of sucrose, 2 percent of cod-liver oil, 8 percent of butterfat, 10 or 12 percent of lard, and enough starch to make 100 percent. Vitamins B and G were supplied extra as Harris yeast vitamin powder.

The deficiency of the low-temperature (r. p.) powder in cystine was readily demonstrated in a 14-day feeding test with eight pairs of rats. In this short period the rat in each pair given the cystine supplement gained more in weight than its pair mate on the unsupplemented diet on the same amount of food and attained a greater body length. The average excess gain by the test rats was 7.37 g, the standard deviation of excess gains was 2.75 g, and the probability that fortuitous factors would have produced so consistent an outcome is only 0.0001, according to Student's (15) probability tables. The average difference in body length was 5.50 mm, the standard deviation of differences 3.94 mm, and the probability of a chance outcome only 0.0038.

That preheated skim-milk (s. p.) powder is also deficient in cystine had been previously demonstrated by Mitchell and Beadles (9), the skim-milk powder used being of this description.

The evidence thus far adduced indicates that the initial drop of about 9 to 11 percent in the nutritive value of skim-milk proteins during drying by prevailing commercial methods must be the result of a destruction of cystine, since cystine is still the amino acid limiting the biological value of commercial skim-milk powders. However, the growth-promoting value of the slightly scorched (r. p.) powder was not improved by a cystine supplement. Eight pairs of rats were used in this test, and at the end of 2 weeks the gains of pair mates were very nearly the same, being exactly equal in 2 pairs, only 1 g apart in 4 pairs, 2 g apart in 1 pair, and 5 g apart in the remaining pair. The rats receiving cystine supplements gained more than their control mates on the same amount of food in only 2 pairs. At this point in the feeding experiment the control rats, previously consuming the unsupplemented diet, were given a supplement of lysine dihydrochloride equal to 0.3 percent of the basal diet. The other rats in each pair continued on the basal diet plus cystine.

Three weeks after this change in plan was put into effect, the rats receiving the lysine supplement had exceeded in gain their pair mates receiving the cystine supplement in all of the 8 pairs and had also attained to greater body lengths in all pairs. The average difference in gain between pair mates was 6.37 g, the standard deviation of differences 2.19 g, and the probability that a purely random com-

bination of factors common to both pair mates would produce as consistent an outcome as this is negligible, amounting to less than 0.0001. The average difference in body length (from nose to root of tail) is 5.25 mm, the standard deviation of differences, 3.23 mm, and the desired probability only 0.0018, again entirely negligible. Body length measurements, somewhat more surely than body weight measurements, are reliable criteria of growth, and in this case demonstrate beyond question that a lysine supplement to the proteins contained in the slightly scorched skim-milk powder increased their growth-promoting value. The complete data of this test will be found in table 10.

TABLE 10.—*Effects of supplements of cystine alone for 2 weeks and of cystine and lysine for 3 weeks on the growth-promoting value of slightly scorched skim-milk powder (r. p.)*

Results of the first 2 weeks of feeding					Results of the last 3 weeks of feeding					
Pair no. and diet or supplement	Total food	Initial weight	Final weight	Gain	Pair no. and supplement	Total food	Initial weight	Final weight	Gain	Body length
Pair 1, females:	g	g	g	g	Pair 1, females:	g	g	g	g	mm
Basal only.....	109	63	87	24	Lysine.....	164	87	118	31	182
Cystine.....	109	58	82	24	Cystine.....	164	82	106	24	173
Pair 2, females:					Pair 2, females:					
Basal only.....	97	56	74	18	Lysine.....	180	74	111	37	175
Cystine.....	97	56	73	17	Cystine.....	178	73	108	35	169
Pair 3, females:					Pair 3, females:					
Basal only.....	77	48	62	14	Lysine.....	142	62	93	31	163
Cystine.....	77	48	61	13	Cystine.....	144	61	88	27	160
Pair 4, females:					Pair 4, females:					
Basal only.....	85	46	62	16	Lysine.....	148	62	91	29	163
Cystine.....	85	47	64	17	Cystine.....	148	64	86	22	159
Pair 5, females:					Pair 5, females:					
Basal only.....	103	51	74	23	Lysine.....	186	74	114	40	173
Cystine.....	103	49	67	18	Cystine.....	186	67	99	32	167
Pair 6, females:					Pair 6, females:					
Basal only.....	102	53	70	17	Lysine.....	170	70	103	33	168
Cystine.....	102	54	69	15	Cystine.....	170	69	93	24	166
Pair 7, females:					Pair 7, females:					
Basal only.....	108	53	75	22	Lysine.....	184	75	112	37	176
Cystine.....	108	51	73	22	Cystine.....	184	73	102	29	165
Pair 8, females:					Pair 8, females:					
Basal only.....	96	47	67	20	Lysine.....	204	67	115	48	172
Cystine.....	96	48	69	21	Cystine.....	204	69	111	42	171

The sample of scorched skim-milk powder (r. p.), by similar paired-feeding tests, was found not to be improved in growth-promoting properties by supplements of cystine, histidine, or tryptophane. However, when supplemented by lysine, this sample, like the slightly scorched sample, exhibited a clear improvement in its power to promote growth. Only four pairs of rats were used in this test, with the results summarized in table 11. In each pair, the rat receiving the lysine supplement gained the faster and attained the greater body length. In spite of the fact that only four pairs of rats were used, the results are quite highly significant. Thus, the average difference in gain between pair mates was 7.75 g, the standard deviation of differences 4.09 g, and the probability of a fortuitous outcome, only 0.026. The average difference in body length was 5.75 mm, the standard deviation of differences 2.86 mm, and the desired probability only 0.020.

TABLE 11.—*Effect of a lysine supplement on the growth-promoting value of the proteins of scorched skim-milk powder (r. p.)*

Pair no. and diet or supplement	Total food	Initial weight	Final weight	Gain	Body length
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Millimeters</i>
Pair 1, females:					
Basal only.....	310	59	89	30	161
Lysine.....	307	57	88	31	163
Pair 2, females:					
Basal only.....	359	61	94	33	163
Lysine.....	359	61	105	44	172
Pair 3, females:					
Basal only.....	278	49	72	23	153
Lysine.....	278	49	80	31	157
Pair 4, females:					
Basal only.....	285	55	72	17	154
Lysine.....	285	55	83	28	162

From this series of paired-feeding experiments it appears that the initial stages of the destruction of milk proteins by heat, occurring during the drying according to prevailing commercial processes, involves, and is the direct result of, a destruction of cystine, but that the later stages, from the initiation of perceptible scorching to the production of a thoroughly scorched product, involve a more rapid destruction of lysine than of cystine. This is a finding of considerable practical importance, since the value of milk proteins in supplementing the proteins of the cereal grains is the result of the deficiency of the latter in lysine and of the presence in the former of abundant proportions of this amino acid. Hence, scorched skim-milk powders, merchandisable only for animal feeding, would not possess this supplementing capacity.

CONCLUSIONS

The proteins of milk are very sensitive to the intensities and durations of heat treatment employed in commercial drying. However, it is possible to dry skim milk with commercial equipment without appreciably affecting its energy value or the nutritive value of its proteins. In the preparation of choice commercial roller-process powders, or of preheated spray-process powders, the biological value of the protein is lowered about 8 percent (from 90 to 82), although its digestibility is not appreciably affected. If preheating is omitted in the spray-drying process this reduction in nutritive value of the milk proteins does not occur. Since cystine is the amino acid limiting the biological value of the proteins of choice commercial roller-process powder and preheated spray-process powder, as well as of the original milk, it may be concluded that this initial decline in biological value of milk proteins is due to a partial destruction of cystine.

As the temperature of drying in the roller process is increased until perceptible scorching occurs the biological value of the milk proteins is rapidly lowered from 82 to 70 or less. The scorched products thus obtained are no longer benefited by cystine additions, but they do respond to lysine additions in increased nutritive value of their proteins. Hence the rapid change in milk proteins at the scorching point (or earlier) is primarily a result of the destruction of lysine. Such products, therefore, are of no value as supplements to the proteins of cereal grains. The digestibility of the milk proteins is also lowered at the scorching point in the roller-drying process, the extent

of lowering increasing more rapidly with the degree of scorching than the reduction in biological value.

However, even with extreme scorching, the net-energy value appears to be but slightly (if at all) affected. Apparently the protein disintegration occasioned by scorching conditions does not impair appreciably the value of the protein as a source of energy to the body.

The solubility of the total solids and the nitrogen of dry skim-milk powders is greater for spray-process than for roller-process powders and decreases in the latter with the intensity of the drying conditions. The spray-process powders gave a higher percentage of brightness than the roller-process powders when colors were analyzed, and in general brightness decreased as the heat increased. These solubility differences and color differences, however, are not reliable criteria of the changes occurring in the nutritive value of the proteins.

LITERATURE CITED

- (1) BLOCK, R. J., JONES, D. B., and GERSDORFF, C. E. F.
1934. THE EFFECT OF DRY HEAT AND DILUTE ALKALI ON THE LYSINE CONTENT OF CASEIN. *Jour. Biol. Chem.* 105: 667-668.
- (2) BOAS FIXSEN, M. A., and JACKSON, H. M.
1932. THE BIOLOGICAL VALUES OF PROTEINS. IV. THE BIOLOGICAL VALUES OF THE PROTEINS OF WHEAT, MAIZE AND MILK. *Biochem. Jour.* 26: [1923]-1933.
- (3) CHICK, H.
1926. SOURCES OF ERROR IN THE TECHNIQUE EMPLOYED FOR THE BIOLOGICAL ASSAY OF FAT-SOLUBLE VITAMINS. *Biochem. Jour.* 20: [119]-130, illus.
- (4) COWARD, K. H., KEY, K. M., MORGAN, B. G., and CAMBDEN, M.
1929. THE INFLUENCE OF DIFFERENT SAMPLES OF "CASEIN" ON VITAMIN TESTS. *Biochem. Jour.* 23: [913]-920, illus.
- (5) GOLDBLATT, H., and MORITZ, A. R.
1927. THE EFFECT OF HEAT AND OXIDATION ON THE NUTRITIVE VALUE OF A PROTEIN. *Jour. Biol. Chem.* 72: 321-326.
- (6) GREAVES, E. O., and MORGAN, A. F.
1934. NUTRITIVE VALUE OF RAW AND HEATED CASEIN WITH AND WITHOUT ADDED AMINO-ACIDS. *Soc. Expt. Biol. and Med. Proc.* 31: 506-507.
- (7) MITCHELL, H. H.
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. *Jour. Biol. Chem.* 58: 873-903.
- (8) ———
1924. THE BIOLOGICAL VALUE OF PROTEINS AT DIFFERENT LEVELS OF INTAKE. *Jour. Biol. Chem.* 58: 905-922.
- (9) ——— and BEADLES, J. R.
1930. THE PAIRED-FEEDING METHOD IN NUTRITION EXPERIMENTS AND ITS APPLICATION TO THE PROBLEM OF CYSTINE DEFICIENCIES IN FOOD PROTEINS. *Jour. Nutrition* 2: 225-243.
- (10) ——— and CARMAN, G. G.
1926. THE BIOLOGICAL VALUE OF THE NITROGEN OF MIXTURES OF PATENT WHITE FLOUR AND ANIMAL FOODS. *Jour. Biol. Chem.* 68: 183-215.
- (11) MIYAWAKI, A., KANAZAWA, K., and KANDA, S.
1932. THE DIGESTIBILITY OF PROTEIN OF DRIED MILK MANUFACTURED BY DIFFERENT PROCESSES. *Jour. Dairy Sci.* 15: 62-70.
- (12) MORGAN, A. F.
1931. THE EFFECT OF HEAT UPON THE BIOLOGICAL VALUE OF CEREAL PROTEINS AND CASEIN. *Jour. Biol. Chem.* 90: 771-792.
- (13) NEVENS, W. B., and SHAW, D. D.
1933. THE EFFECT OF DAIRY MANUFACTURING PROCESSES UPON THE NUTRITIVE VALUE OF MILK. II. THE APPARENT DIGESTIBILITY OF FRESH WHOLE MILK AND OF POWDERED WHOLE MILK. *Jour. Nutrition* 6: 139-150.

-
- (14) SHIFTAN, H.
1932-33. ÜBER DIE BIOLOGISCHE WERTIGKEIT DES FUTTEREIWEISSES BEI WACHSENDEN SCHWEINEN. *Wiss. Arch. Landw. Abt. B, Arch. Tierernährung u. Tierzucht* 8: 212-245.
- (15) STUDENT.
1908. THE PROBABLE ERROR OF A MEAN. *Biometrika* 6: 1-25, illus.
- (16) WESSON, L. G.
1932. A MODIFICATION OF THE OSBORNE-MENDEL SALT MIXTURE CONTAINING ONLY INORGANIC CONSTITUENTS. *Science (n. s.)* 75: 339-340.
- (17) WRIGHT, N. C.
1932. FACTORS AFFECTING THE SOLUBILITY OF MILK POWDERS. I. THE EFFECT OF HEAT ON THE SOLUBILITY OF MILK PROTEINS. *Jour. Dairy Research* 4: [122]-141, illus.

EVALUATION OF LENGTH MEASUREMENT IN AN EXPERIMENT WITH APPLE TREES¹

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INTRODUCTION

Adequate measurement of experimentally induced differences in fruit trees is rarely attained. Yields obviously constitute the results of greatest ultimate interest, but unsupported yield records usually tell little about the precise mechanism by which the yields were secured and consequently have limited value as indicators of the general applicability of the results. Wood-growth measurements may represent in some degree what the tree has done, but leaf surface may be a better indicator of what it is about to do. Quantitative measurements of any sort, whether of wood growth, spur number, or leaf surface, present incomplete pictures since they do not well represent distribution, and the experienced fruit grower or investigator may have some justification for his belief that his eyes will tell him more about a tree than any amount of data will reveal.

Quantitative measurements, nevertheless, are obviously necessary, and the realization of their imperfections should only stimulate study leading to their improvement. Though no one index figure may ever be attained that will express all the performance and potentiality of a tree, various standards may be used to represent it at various stages, and each should be improved as far as possible.

The best measure of accomplished vegetative growth is generally considered to be the weight of dry matter produced. Since complete determination of this manifestly terminates an experiment, some sort of measurements of size seems to be the best means of gaging growth that is possible in many cases. Trunk girth measurements are of undoubted value, particularly in trees not yet bearing, but have inherent limitations in the relatively great importance of small errors in measurements as well as difficulties of execution due to trunk excentricity, sunscald, wounds, proximity of scaffold limbs, etc. Consequently length has been widely used.

In actual application, however, length measurement may present complications. On large trees enough analogous shoots may be chosen to yield a constant average, i. e., one that is not modified by duplicate sampling. Any comparison of trees made by this standard is predicated on the assumption, usually not established, that the number of comparable shoots is identical. For a short time after experimental differences, as for example by soil treatments, have been produced, this assumption is justifiable; in pruning experiments of any duration and after several years of soil treatments it is questionable. The possible fallaciousness of mean growth is illustrated by some measurements on young apple trees, where all growths of 10 cm. or over, in 1934 were recorded. Two trees had identical means, 50.2 cm, but on one this was an average of 20 shoots, while, on the other, 29 shoots

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contributed to the average; the respective total growths (for 1934) were 1,004 and 1,456 cm. This being true, its converse may be anticipated; the writers' records show another pair of trees with total growths of 833 and 836 cm, respectively, representing the total lengths of 18 shoots in the former and of 40 in the latter, with consequent respective averages of 46.3 and 20.9 cm.

Total length is an unreliable measure of growth, if weight is a good measure. Weights of 1-year shoots are not directly proportional to their lengths, where differences of much consequence exist. The mean fresh weights per linear centimeter in dormant 1-year shoots of Red Canada (Steele Red) (samples of 25 to 30 specimens each) varied with length, as follows: 25 cm, 0.09 g; 35 cm, 0.12 g; 45 cm, 0.14 g; 55 cm, 0.17 g; 65 cm, 0.21 g. In the pair of trees just mentioned, having 833 and 836 cm, respectively, of total growth in 1934, the actual weight of new wood in the former was 34 percent greater than in the latter. In the pair of trees mentioned first, the one with 1,456 cm total growth had not produced merely 45 percent more weight than the one with 1,004 cm—it had virtually doubled it. Even more surprisingly, simple total-length measurements can make the smaller appear to be the larger, as will be shown presently.

Greater accuracy in application of length measurements might be expected from the use of a scale which made allowance for an increasing weight per unit of length as length increases. The applicability of any graduated scale of this sort obviously depends in considerable degree on the variability of the material. An account of the construction of two graduated scales and their application to specimens of known weight and to trees under experimental treatment follows.

MATERIAL AND METHOD

The material used was taken from Red Canada apple trees in their second and third seasons in the orchard of the Michigan Agricultural Experiment Station at East Lansing. These trees are pruned only to secure proper spacing of scaffold limbs and receive little or no tipping back; consequently the prunings contain the full season's growth of the shoots removed. While these trees still appeared completely dormant in March 1935, the regular pruning was done, and the prunings from each tree were tied in a bundle and tagged. The total weight and the weight of the 1934 wood were recorded in the laboratory for each bundle, and each shoot was measured. All shoots of 1934 growth were then assembled, sorted into length classes (10-cm intervals), the weight of each class was determined, and the component shoots were counted. The means thus secured constitute the scale here called graduated scale A.

The values on this scale, when graphed, fitted rather well the equation—

$$X^2 = 3.1 Y$$

where X equals the length in centimeters and Y the weight in grams. Individual weights and lengths of 1,428 of these shoots showed a straight-line correlation coefficient of 0.32 ± 0.016 . Computed from deviations from the values in the equation $X^2 = 3.1 Y$, the index of correlation² was 0.93, indicating the closer fit of the parabola.

$$r_{12} = \sqrt{1 - \frac{S_y^2}{S_x^2}}$$

Since the weight values assigned in scale A change abruptly and considerably (table 1) another scale, called B, assigning values to each centimeter increase in length, was constructed by smoothing 1,428 records of individual weights and lengths. These, when tabulated for each centimeter of length, beginning with 21 cm, exhibited rather striking uniformity. For example, the probable error of weights of shoots 25 cm long was 3.11 percent of the mean; for shoots 35 cm long it was 2.38 percent; and for 65 cm, 1.89 percent. The values used in the scale were moving averages, smoothed slightly; for values below 21 cm scale A was used.

To bring simple length measurements into a denomination comparable with the graduated scales, the mean weight per centimeter of all shoots studied, 0.17022 g, was shortened to 0.17, and all lengths were multiplied by this factor; this constitutes the simple scale.

After length and fresh weight had been determined, all wood was dried to constant weight in an oven at 100° C. The dry weights thus determined bore a uniform relationship to the fresh weights and showed nothing new. The studies here reported are, therefore, confined to fresh-weight figures.

To try the accuracy of the three scales, weights of prunings from each tree were then calculated by each scale and compared with the actual weights previously recorded (table 2).

TABLE 1.—*Values used in computing weight of shoot from length by scales A and B and by simple weight*

Graduated scale A		Graduated scale B		Simple weight
Length	Weight	Length	Weight	
<i>Centimeter</i>	<i>Grams</i>	<i>Centimeter</i>	<i>Grams</i>	<i>Grams</i>
0-10	0.4			
11-20	1.3	20	1.3	3.4
21-30	2.3	25	2.2	4.3
		30	3.0	5.1
31-40	4.2	35	4.2	6.0
		40	5.3	6.8
41-50	6.6	45	6.3	7.7
		50	7.6	8.5
51-60	9.5	55	9.3	9.4
		60	11.1	10.2
61-70	13.0	65	12.9	11.1
		70	15.1	11.9
71-80	18.0	75	18.1	12.8
		80	21.1	13.6
81-90	23.5	85	24.8	14.5
		90	28.3	15.3
91-100	32.4	95	31.4	16.2
		100	34.6	17.0
100-110	40.5	105	37.4	17.9
		110		18.7

Individual records from four pairs of trees are shown in table 2 as illustrative of the varying significance of length measurements. The prunings representing trees A and B, judged by simple length measurements, were virtually identical; in actual weight there was a difference of nearly 46 percent. Between the prunings from trees C and D there was a length difference of 41 percent, while the weight difference was less than 1 percent. Tree E's prunings were more than 14 percent greater in length, and 14 percent less in weight, than those of

tree F. Similarly the shoots removed from tree G were 14 percent longer than those of tree H, but weighed 31 percent less. Had these shoots remained on the trees and been compared simply on the basis of shoot length, some very real difference would have been obscured. The graduated scales gave greater accuracy than length measurements in seven of the eight comparisons.

In some cases the graduated scales give weight indications at considerable variance from the actual. In a large number of determinations, however, the results secured by these methods approximate the actuality rather well. In the 155 cases for which data are available, scale A gave closer approximation than the simple scale in 69.7 percent of the cases, and scale B was more accurate than the simple scale in 69.4 percent. Deviations as calculated from the actual weights by the graduated scales were about half those secured by the simple scale, as shown in table 3.

TABLE 2.—*Effect of length of shoot on weight of wood produced*

Item	Data for tree—							
	A	B	C	D	E	F	G	H
Total growth.....centimeter	507	503	463	328	800	698	647	566
Shoots of indicated length in centimeters:								
10-20.....number	5		2		9		1	
21-30.....do	2		3		1		1	
31-40.....do	7	1	7	3	4		4	1
41-50.....do	3	1	2	3	5	2	5	
51-60.....do		3			2	3	3	4
61-70.....do		4		1		3	1	2
71-80.....do					1	1		1
81-90.....do					1	2		
91-100.....do								1
Total shoots.....	17	9	14	7	23	11	15	9
Calculated weight by—								
Scale A.....grams	50.7	91.7	52.7	45.4	123.2	145.7	95.1	118.6
Scale B.....do	60.6	88.1	53.8	50.3	130.4	151.0	95.0	121.5
Simple.....do	86.2	85.5	78.7	55.8	136.0	118.7	110.0	96.2
Actual weight.....do	63.8	93.0	52.5	52.0	124.1	144.6	96.0	136.6

TABLE 3.—*Deviation from the actual of weights calculated from length measurement by use of graduated scales and of average weight*

Item	Scale A		Scale B		Length (centimeters)×0.17	
	Cases	Mean deviation	Cases	Mean deviation	Cases	Mean deviation
Deviations.	Number	Grams	Number	Grams	Number	Grams
Above actual.....	72	3.75	67	4.23	103	8.45
Below actual.....	83	5.75	96	5.98	51	15.82
Total.....	155		163		154	
Error.....percent		4.8		5.3		10.8
Percentage error.....		7.7		8.4		17.2

¹ No deviation in 2 cases and in 1 case, respectively.

For the entire group the several coefficients of variability of prunings, tree by tree, were: Length, 0.63; actual weight, 0.74; scale A, 0.71; scale B, 0.74.

Both graduated scales underestimate weight more frequently than they overestimate it, and the margin of error is greater in underestimation than in overestimation. Comparison of the 9 cases of greatest overestimation by scale A with the 10 cases of greatest underestimation by this method shows that overestimation occurred predominantly in cases involving longer growths, underestimation in cases in which shorter growths were more numerous. In the cases of greatest overestimation the percentage of shoots measuring under 31 cm was 12.8; in those of greatest underestimation it was 25.5; in overestimation the percentage of shoots over 70 cm was 20.2, while in underestimation it was 7.8.

Whether the pruning, which was done solely to secure proper spacing of scaffold limbs, removed samples which were representative, and whether the differences found in prunings would be paralleled if all growths, pruned and unpruned, were considered, is answered affirmatively by the data (table 4). The overturnings in relative standings here are similar to those shown in the prunings.

TABLE 4.—*Relative rankings of trees on bases of measured length and calculated weight of all 1934 growths over 9 cm in length*

Tree	Length growth	Calculated weight	Relative rank for—		Frequency distribution of shoots of length indicated											
			Length	Weight	10 cm	11-20 cm	21-30 cm	31-40 cm	41-50 cm	51-60 cm	61-70 cm	71-80 cm	81-90 cm	91-100 cm	Total	
	<i>Centimeters</i>	<i>Grams</i>													<i>Number</i>	
A.....	882	99	1	12	2	25	7	3	3	—	—	1	—	—	41	
B.....	878	146	2	2	1	5	5	2	1	3	5	1	—	—	23	
C.....	876	140	3	3	—	3	—	1	7	6	2	—	—	—	19	
D.....	870	104	4	10	1	18	7	4	4	2	—	—	—	—	36	
E.....	869	117	5	7	2	6	1	9	6	3	—	—	—	—	27	
F.....	843	135	6	6	—	1	1	4	5	4	2	—	—	—	17	
G.....	839	69	7	13	8	41	5	—	—	—	—	—	—	—	54	
H.....	838	112	8	9	—	3	8	5	1	5	1	—	—	—	23	
I.....	837	102	9	11	4	20	11	1	2	1	—	1	—	—	40	
J.....	834	137	10	5	—	5	—	1	4	8	2	—	—	—	18	
K.....	823	116	11	8	—	3	—	—	4	2	2	—	—	—	21	
L.....	821	162	12	1	—	1	1	1	2	4	4	1	—	1	15	
M.....	803	138	13	4	—	—	—	2	2	9	1	1	—	—	15	

Finally, the question must be considered whether compensating error may not, in comparisons involving fair numbers of trees, offset the differences appearing in comparisons involving a few trees. In some cases it plainly does offset these differences. For example, all trees (27), making 1934 growths between 600 and 699 cm and all (17) making growths between 800 and 899 cm were compared. In average length the latter group exceeded the former by 30.3 percent; in the calculated weight the difference was 31.6 percent in the same direction. Likewise, a lot of 24 trees on one interstock, in their second year in the orchard, exceeded a lot of 44 trees worked directly on seedlings by 30 percent in length growth and by 26.5 percent in weight; in neither case was the difference over three times the probable error of the difference.

On the other hand, rather important differences appear in another group, a year older, in which the magnitude of relationships indicated by the two standards shifts (table 5). There is no change of consequence in ranking, but the change in degree of difference is considerable. Lot A's superiority over lot D is increased, while lot B's margin over lot D, of doubtful statistical significance on the basis of length measurements, is further reduced by reference to the weight basis.

TABLE 5.—Comparison of 1934 growth of lots of Red Canada apple trees on bases of observed length and of calculated weight

Lot	Trees	Length		Weight		Percentage of lot D	
		Mean per tree	Probable error	Mean per tree	Probable error	Length	Weight
	Number	Centimeters	Centimeters	Grams	Grams		
A.....	19	1,373	105.5	223	18.6	161	169
B.....	17	1,032	97.8	140	16.4	121	107
C.....	37	957	86.0	139	9.7	112	106
D.....	19	855	85.2	131	14.4		
E.....	16	847	85.0	132	14.2	99	101

DISCUSSION

The greater accuracy attained by interpreting growth in terms of weight seems to warrant its consideration in studies involving length measurements, at least in young trees. In some, perhaps in most, cases, little change will be made from results recorded as simple length measurements, but the other cases are likely, for this reason, to be important enough to repay examination of all. In mature trees it seems unlikely to be of great usefulness, since other standards, such as spur production, spur performance, and yield are more valuable. As a means of comparing response of different varieties it seems promising. In interpretation of growths in the nursery it is particularly valuable.

Obviously, this method can be applied only to wood of most recent growth. The weight of 2-year-old wood is affected by the growth made in two seasons; in older wood the weight is a still more complicated product.

The labor involved in use of the graduated scale need not be much greater than that involved in simple length measurements. Once the values are established for each variety and condition, a meter stick can be recalibrated to read directly in grams and the measurements recorded directly, with no increase whatever in labor of recording or calculation.

THE DETERMINATION AND IMPORTANCE OF THE CONDITION OF THE FIRM ALBUMEN IN STUDIES OF EGG-WHITE QUALITY¹

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INTRODUCTION

The demand by consumers for eggs of high quality, as shown by market surveys, is reflected in the increasing stress that is being laid on this factor and its measurement in experimental work conducted on poultry. While candling is the basis for establishing the market value of eggs, it is too indefinite to be of more than superficial value in research. Therefore, it has been necessary to study the component parts of the egg. Various investigators have used one or more of the following measurements as criteria of egg quality exclusive of nutritive value: The size of egg; the thickness or strength, porosity, texture, and color of the shell; the color, dimensions, condition, flavor, and odor of the yolk; and the color, relative volume or weight of the various layers of the albumen, and condition of the firm albumen.

The importance of most of these factors has been generally accepted by the majority of investigators. Most workers have used the relative proportions of firm and thin albumen as the sole criterion of albumen quality. The determination of the observed condition, or firmness, of the firm albumen, however, has not received proper recognition. The term "firm albumen" is used throughout this paper to refer to the "true thick albumen" as defined by Sharp (17)³ unless otherwise specifically noted.

It is generally agreed that consumers prefer an egg for table use which holds together well and cooks evenly throughout. The ability of an egg to meet these requirements depends not only upon the quantity of firm albumen but also upon its firmness, body, or condition. There is also the strong probability that this condition of the firm albumen plays an important part in determining the candling properties of the egg, particularly in controlling the apparent mobility, or swing, of the yolk (10). On the other hand, Almquist (1) has found little correlation between the quantity of firm albumen and the apparent mobility of the yolk. Pennington et al. (14) found no relationship between the percentage of thick white and either the candled grade or the yolk index. The reason that no measure of the condition of firm albumen has been generally used in studies of egg quality is undoubtedly that the various physicochemical components which constitute it have not yet been established. However, this is no justification for ignoring an estimation of a factor which plays such

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³ Reference is made by number (italic) to Literature Cited, p. 1136.

an important part in determining the desirability of eggs from the viewpoint of the consumer and probably of the market man.

The nature of the firm albumen has been subjected to some investigation. It appears unlikely from the work of Almquist and Lorenz (4) and of Bronkhorst (6) that the difference between firm and thin albumen is entirely chemical. The findings of St. John and Green (15) and of Almquist and Lorenz (3) strongly suggest that the difference is chiefly a physical one. The latter authors believe that the structure of the firm albumen is due to fibers of ovomucin. Later, McNally (12) and Almquist, Givens, and Klose (2) showed that the firm albumen contains more ovomucin than the other layers. The findings of the latter authors indicated that photometric methods might be used to detect differences due to the effect, presumably of ovomucin, upon the transmission of light. Such measurements, however, must be correlated with the condition of the firm white as noted by observation. Such an observation necessitates a fixed score capable of reproducible results and of universal application.

A method of scoring the condition of the firm albumen was first used by Sharp (16), but he has only recently described and partially illustrated it (17). It is the purpose of this paper to illustrate this method more completely, to present further evidence of its importance, and to extend preliminary data presented by the authors (19) showing that there is not necessarily a correlation between the condition and the quantity of the firm layer.

EXPERIMENTAL METHODS

In the first part of this study, photographs were taken to illustrate the score for the condition of the firm albumen. The pictures shown in figure 1 were all taken of freshly laid eggs from pullets of the Cornell strain of Single Comb White Leghorns but represent conditions of the firm albumen encountered in both fresh and held eggs. The scores run from 1.0 to 5.0, as was suggested by Sharp, with intervals of 0.5. In using this method, it was soon found that the scores could be determined accurately by intervals of 0.25. Both top and side (silhouette) views were taken of each egg to illustrate the way the albumen stood up and was distributed around and over the yolk. Each egg illustrates the lower limit for its group range; thus the egg marked 1.0 is the poorest egg that may be scored 1.0. The egg illustrating 5.0 is the lowest score, since no thick albumen is discernible.

The two chief factors in this observation are the outline of the firm albumen viewed from the top, and the outline viewed from the side. The firm albumen in an egg with the best score is concentrated around and over the yolk and tends to occupy the least possible area on the plate. From the top view its outline appears to be ovoid. As the score increases, the firm white spreads and the outline tends to become more irregular. That of 2.5 is generally the first to show this markedly. In this case, a definite tendency toward a rupture of the firm albumen is noticeable. The firm layer of an egg with the score of 3.0 is usually about to rupture, or has just ruptured, allowing the inner thin albumen to mix with the outer thin albumen. From the side view, the outline shows no distinct break in sweep between the yolk and the firm albumen in the best egg. As the score increases, the firm white flattens and spreads out and the angle between the yolk

and albumen appears and becomes sharper until it approaches 90°. This occurs at about the time of rupture of the firm-albumen sac.

The score of the observed condition of the firm albumen considers the apparent thick white described by Sharp (17). This includes both the true thick and the inner thin albumen up to the point of rupture of the thick-albumen sac. From this point (score 3.0) on, only the true thick albumen, chiefly structural albumen, is scored since the inner thin layer has escaped.

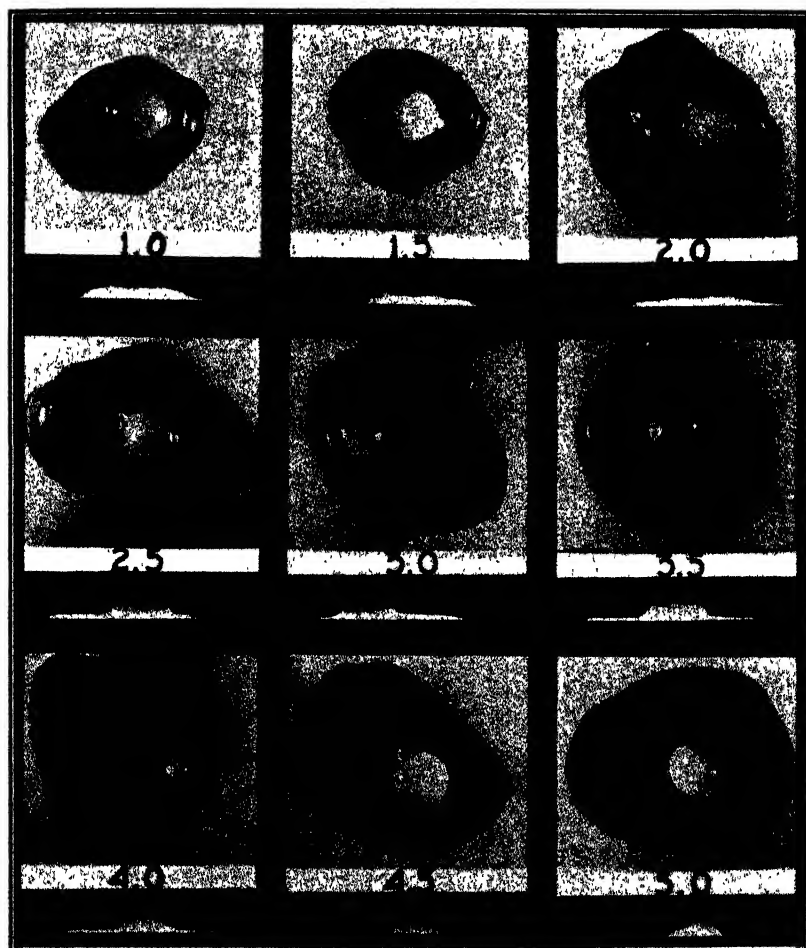


FIGURE 1.—Freshly laid pullet eggs of the Cornell strain of Single Comb White Leghorns scoring 1.0 to 5.0 on the scale suggested by Sharp, showing intervals of 0.5. The eggs shown represent group minima.

In a few instances in which further definition is desirable in exactly placing the score of an egg, the so-called "body" or consistency of the firm albumen may be further determined. This has been done by pouring the firm albumen slowly over the edge of a container and noting its plasticity while retaining the yolk in the container. When quantitative measurements of the albumen are desired, they may be made according to the Sharp (17) method of separation by noting the

difficulty with which the firm albumen enters the pipette, or according to the Lorenz and Almquist method (11) by noting the apparent plasticity in transferring the egg from the scoring dish to the screen. By observation with candling, using New York State retail grades (5), grade Fancy eggs generally score from 1 to 2 when broken out, grade A from 2 to 3, grade B from 3 to 4, and grade C from 4 to 5, but with considerable overlapping.

In the second part of this study, a comparison was made between observed condition of the firm albumen and those factors in candling which would seem to be most closely correlated with albumen condition, in order to determine the relationship between the score for observed condition of the firm albumen and candling quality. A total of 199 eggs was used. These were obtained from several up-State New York City markets and included some fresh eggs from the experiment station flock. Four experienced candlers scored these eggs, and their results were averaged. There was quite regular agreement among the candlers as to the scores for the various factors used. Yolk visibility was scored from 1, for practically invisible yolk shadow, to 4, for a plainly visible yolk shadow. Yolk mobility was scored from 1, for very slight mobility, to 3, for freely mobile yolk shadow. The grades used were from 1 to 8, based upon the New York State retail grades for eggs as described in detail elsewhere (5). Eggs were scored as being high or low within each of the New York State grades Fancy, A, B, and C; thus making a complete score of eight grades. No inedible eggs, as detected before the candle, were used. The eggs were opened immediately after candling and were scored for observed condition of the firm albumen and for yolk color as hereinafter described.

In the third part of this investigation, correlations were made between the relative volumes of the three principal layers of albumen obtained as previously briefly described (19) and the observed condition of the firm albumen. This was done with data obtained in routine studies in this department on the effect of breeding and feeding upon interior egg quality.

All of the 4,796 eggs studied were from the Cornell strain of Single Comb White Leghorn pullets. All of them were examined on the day that they were laid. Each was carefully broken into a Petri dish, preferably one of 15 cm diameter. If the firm albumen was unruptured, the outer layer of thin albumen was removed into a graduated cylinder by means of a 25-cc pipette. The pipette had a bore of approximately 4 mm, with the tip broken off and the broken end fire-polished. The observed condition of the firm albumen was then noted. Next, this was torn to allow the inner layer of thin albumen to escape. The firm layer was removed from the side opposite the rupture by drawing it into the pipette and was placed in another graduated cylinder. The chalazae and the removable portions of the inner layer of thick albumen, usually very slight, were included in this fraction. Finally, the inner thin layer was removed to a third cylinder. By careful manipulation, accurate and rapid determinations may be made in this manner. The use of moistened cylinders, dishes, and pipettes reduces the error of drainage and speeds up the work.

The yolk was then transferred to a smaller, perfectly flat-bottomed Petri dish for color determination (by the Sharp standards), for yolk-

index measurements (18), and, finally, for weight determination. When the firm albumen was found to be in the naturally ruptured state, the observed condition was taken first, and then the firm albumen and finally the combined layers of thin albumen were removed.

EXPERIMENTAL DATA AND DISCUSSION

The results showing the means and correlations obtained between the candlers' score and the opened egg score of the factors studied are given in table 1. The mean observed condition of these eggs was considerably below that of 1.76 found for fresh eggs as shown in table 2. In every case, a significant positive correlation was found between the observed condition of the firm albumen and the yolk shadow visibility, yolk shadow mobility, and candlers' grade. This, therefore, indicates that the condition of the firm albumen is closely related to those factors used in determining the candling quality of the egg. In contrast to this is the report of Almquist (1) that there is no correlation between the quantity of firm albumen and the apparent mobility of the yolk, and the report of Pennington et al. (14) that there is no relationship between the quantity of firm albumen and the candled grade.

TABLE 1.—Correlations between candlers' score and opened-egg score of certain factors

Factors correlated	Mean	$r \pm S.E.$
Observed condition of firm albumen	2.45	-----
Correlation of observed condition of firm albumen with—		
Yolk visibility (1=none to 4=plainly)	1.85	$+0.504 \pm 0.054$
Yolk mobility (1=none to 3=freely)	1.77	$+ .543 \pm .051$
Candlers' grade (1 to 8)	3.66	$+ .583 \pm .048$
Actual yolk color (00 to 110) sharp standards	58	
Correlation of actual yolk color with yolk visibility (1=none to 4=plainly)	1.85	$+ .443 \pm .057$

TABLE 2.—Correlations between the observed condition of firm albumen and the volume of the various layers of albumen

Condition of firm-albumen sac	Eggs	Factors correlated	Mean	$r \pm S.E.$
	<i>Number</i>			
Not ruptured....	4,531	Observed condition of firm albumen	1.76 \pm 0.27	-----
		Correlation of observed condition of firm albumen with—		
		True firm albumen	53.7	$+0.020 \pm 0.015$
		Outer thin albumen	24.4	$- .015 \pm .015$
		Inner thin albumen	21.9	$- .006 \pm .015$
Ruptured.....	265	Observed condition of firm albumen	3.38 \pm .47	-----
		Correlation of observed condition of firm albumen with—		
		True firm albumen	55.2	$- .212 \pm .059$
		Total thin albumen	44.8	$+ .178 \pm .059$
		Observed condition of firm albumen	3.37 \pm .47	
Do. ¹	261	Correlation of observed condition of firm albumen with—		
		True firm albumen	55.8	$- .061 \pm .062$
		Total thin albumen	44.2	$+ .022 \pm .062$

¹ 4 abnormal eggs eliminated.

It is interesting to note that the correlation between actual yolk color and visibility of the yolk shadow is significant but not as high

as that between condition of firm albumen and visibility of the yolk shadow. Data obtained with this group of eggs support the opinion of some investigators that the firm albumen is probably of as much or more importance in determining visibility of the yolk shadow than is the actual color of the yolk.

The averages and distribution obtained in the third part of this study for the observed condition of the firm albumen and the proportions of the three chief layers of albumen are shown in table 2 and figures 2 and 3, respectively. The proportions of the various layers of

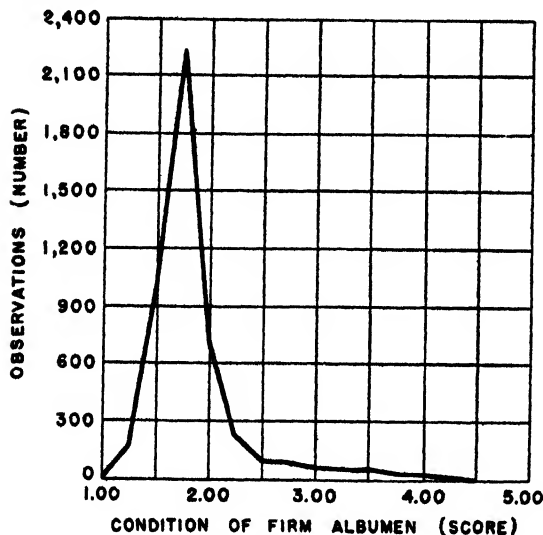


FIGURE 2.—Distribution of the scores for the observed condition of the firm albumen of 4,790 eggs less than 1 day old.

albumen are in general agreement with those obtained by Almquist (1). The amount of firm albumen, however, is considerably less than that reported by Card and Sloan (7) and by North (13). It is interesting to note that the amount of firm albumen was slightly greater in the eggs of poor condition in which the firm albumen sac was ruptured. This apparent increase has also been observed, despite a decided increase in score of condition, in eggs from individuals producing eggs of

known interior quality which were stored for periods of from 7 to 10 days. The firm-albumen sac was unruptured in 4,531 of the eggs and permitted the determination of correlations of observed condition of firm albumen with the three main layers of albumen. The results, given in table 2, show that there was not the least correlation between the observed condition of the firm albumen and the proportion of the firm, outer thin, or inner thin layers of albumen.

In the 265 eggs in which this sac was ruptured, the inner thin layer was mixed with the outer thin, hence these eggs could not be included in the larger group. There were slight, though insignificant, correlations between the observed condition of the firm albumen and the proportion of firm albumen and the proportion of total thin albumen, respectively. These slight trends, however, were due to four eggs which possessed an abnormally small amount, or in one case, none, of the firm albumen, and correspondingly large amounts of thin albumen. With these extraordinary observations eliminated, no correlations existed (table 2).

These data present conclusive evidence that the proportion of true firm albumen, used by many investigators as the sole criterion of

albumen quality, is actually in no way related to the firmness, or condition, of the firm albumen. Furthermore, the proportion of inner or outer thin albumen is likewise no indication of the condition of the firm layer.

These findings are in general agreement with the observations of Sharp (17) on storage eggs. The two studies cannot be directly compared, however, since he used what he called "apparent thick white", whereas the present study refers to what he defined as "true thick white." The fact that the score of the observed condition of the firm albumen decreases during storage (8) and the amount of firm albumen also decreases (9) during storage indicates that a correlation should be found between the observed condition and the proportion of the firm albumen of eggs subjected to any considerable period of storage.

If the findings of Almqvist and Lorenz (3), that the firmness of the firm albumen is due to the presence of ovomucin, and those of McNally (12), that the firm albumen contains considerably more ovomucin than the thin albumen, are substantiated, the quantitative determination of ovomucin might be used as a more exact measure of the condition of this layer of albumen. It has not been shown, however, that the methods of either authors are quantitative or that the amount of ovomucin is related to the quality or the quantity of firm albumen. That they are not quantitative is indicated by the lack of even moderate agreement in their figures. Furthermore, the use of a chemical method is at a distinct disadvantage when compared with a score such as the observed condition, since the large amount of time involved in a chemical analysis prohibits the use of the number of eggs necessary in studies on egg quality.

This score is not proposed to take the place of more accurate means for measuring this condition when they become available. Some measurements of a physical nature are under study here. One of the most promising of these is the height of the apparent firm albumen weighted for the size of egg and expressed in terms of a 2-ounce egg. The score does, however, offer a valuable aid in studies of egg quality in the interim, and it should supplement such measurements as they become available, since it scores the sum of the factors which constitute albumen quality as seen by the consumer.

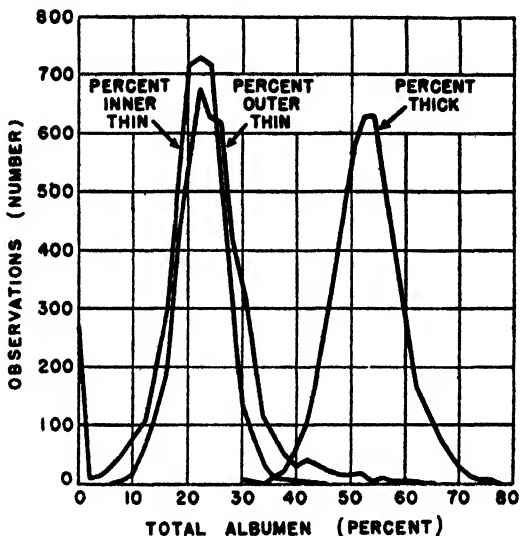


FIGURE 3 —Proportions of the 3 main layers of albumen in 4,796 eggs less than 1 day old.

SUMMARY

A method for determining the observed condition of the firm albumen of eggs is illustrated. Significant correlations were found between the observed condition of the firm albumen and the visibility and the mobility of the yolk shadow, respectively. Thus, as the yolk shadow appeared more visible and mobile in candling, the score of the condition of the firm albumen was poorer. No correlation was found between the observed condition and the percentage of true firm albumen in freshly laid eggs. It is therefore necessary to measure the condition as well as the quantity of firm albumen in the egg.

LITERATURE CITED

- (1) ALMQUIST, H. J.
1933. RELATION OF THE CANDLING APPEARANCE OF EGGS TO THEIR QUALITY. *Calif. Agr. Expt. Sta. Bull.* 561, 31 pp., illus.
- (2) ——— GIVENS, J. W., and KLOSE, A.
1934. TRANSMISSION OF LIGHT BY EGG ALBUMEN. *Indus. and Engin. Chem.* 26: 847-848.
- (3) ——— and LORENZ, F. W.
1932. LIQUEFACTION OF EGG WHITES. CAN IT BE CONTROLLED OR PREVENTED AND CAN WE BREED FOR EGGS WITH FIRM WHITES. *Nulaid News* 9 (10): 10-12, illus.
- (4) ——— and LORENZ, F. W.
1933. THE SOLIDS CONTENT OF EGG WHITE. *Poultry Sci.* 12: 83-89.
- (5) BALDWIN, C. H.
1934. EGGS. NEW YORK STATE RETAIL GRADES AND STANDARDS FOR EGGS WITH RULES, REGULATIONS, AND LAW. *N. Y. Dept. Agr. and Markets Circ.* 482, 15 pp.
- (6) BRONKHORST, J. J.
1933. A PHYSICAL, CHEMICAL AND PHYSIOLOGICAL STUDY OF HIGH AND LOW HATCHING LINES OF SINGLE COMB WHITE LEGHORNS. 110 pp. Ithaca. (Thesis, Ph. D., Cornell Univ.)
- (7) CARD, L. E., and SLOAN, H. J.
1934. THE EFFECT OF DIFFERENT DIETS ON INTERIOR EGG QUALITY. *Poultry Sci.* 13: 313-314.
- (8) HERRINGTON, B. L., and Sharp, P. F.
1934. THE EFFECT OF HOLDING ON THE APPEARANCE OF THE OPENED EGG. *U. S. Egg and Poultry Mag.* 40 (11): 37-39, illus.
- (9) HOLST, W. F., and ALMQUIST, H. J.
1931. MEASUREMENT OF DETERIORATION IN THE STORED HEN'S EGG. *Hilgardia* 6: [49]-60, illus.
- (10) ALMQUIST, H. J., NELSON, B. O., and LORENZ, F. W.
THE EFFECT OF SHAKING ON THE QUALITY OF EGGS. *U. S. Egg and Poultry Mag.* 40 (12): 13-16.
- (11) LORENZ, F. W., and ALMQUIST, H. J.
1934. DETERMINATION OF THE PERCENTAGE OF FIRM WHITE. *U. S. Egg and Poultry Mag.* 40 (11): 30-33, illus.
- (12) McNALLY, E.
1933. RELATIVE AMOUNT OF MUCIN IN THICK AND IN THIN EGG WHITE. *Soc. Expt. Biol. and Med. Proc.* 30: 1254-1255.
- (13) NORTH, M. O.
1934. POULTRY FEEDING, HOUSING AND LIGHTING EXPERIMENTS AT THE WYOMING EXPERIMENT STATION. *Wyo. Agr. Expt. Sta. Bull.* 203, 52 pp., illus.
- (14) PENNINGTON, M. E., TRUE, M. J., RICH, A. D., and KIESS, A. A.
1934. YOLK INDEX AND THICK WHITE IN EGGS GRADED BY CANDLING FOR INTERIOR QUALITY. *U. S. Egg and Poultry Mag.* 40 (5): 43-46, 48, 52, illus.
- (15) ST. JOHN, J. L., and GREEN, E. L.
1930. THE COLLOIDAL STRUCTURE OF EGG WHITE AS INDICATED BY PLASTICITY MEASUREMENTS. *Jour. Rheology* 1: 484-504, illus.

-
- (16) SHARP, P. F.
1929. WHAT ONE WEEK MAY DO TO AN EGG. PROGRESSIVE DETERIORATION IS INDICATED BY THE WEAKENING OF YOLKS. U. S. Egg and Poultry Mag. 35 (6): 14-17, 64, illus.
- (17) ———
1934. THE CONDITION OF THE APPARENT THICK WHITE AS AN IMPORTANT FACTOR IN STUDYING THE QUALITY OF EGGS. U. S. Egg and Poultry Mag. 40 (11): 33-37, illus.
- (18) ——— and POWELL, C. K.
1930. DECREASE IN INTERIOR QUALITY OF HEN'S EGGS DURING STORAGE AS INDICATED BY THE YOLK. Indus. and Engin. Chem. 22: 908-910 illus.
- (19) VAN WAGENEN, A., and WILGUS, H. S., Jr.
1934. OBSERVATIONS ON THE RELATION OF THE PERCENTAGES OF THE DIFFERENT LAYERS OF EGG ALBUMEN TO OBSERVED INTERIOR EGG QUALITY. U. S. Egg and Poultry Mag. 40 (6): 37, 62.

THE DETERMINATION OF SPRAY COVERAGE ON APPLES¹

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The importance of determining the deposit of insecticides on fruit in spray investigations seems to have been underestimated by many workers in this field. Published reports have frequently given comparisons of the effectiveness of spray programs without stating the quantitative and qualitative nature of the deposit of the insecticide. It often happens that an effective insecticide gives poor control simply because very little of it remains on the fruit.

The physical and chemical properties of powdered insecticides differ greatly. The density of calcium arsenate is about $2\frac{1}{2}$ g per cubic centimeter, while that of lead arsenate is nearly 6 g. It cannot be assumed that different insecticides, mixed in the spray tank in the same way, will be deposited in the same quantities on the fruit surface. Yet the weight of the material per 100 gallons of water is frequently the only basis for comparison employed by investigators. Comparisons of these weights give useful economic information, but they should not be relied upon for comparisons of control. Repeated analyses of representative samples of the sprayed fruit to determine the mass of insecticide per unit area seems to be the logical basis of comparison. This paper describes the methods used at the Washington Experiment Station for the determination of arsenical deposit on apples.

Frequently more than a third of the insecticide deposit on an apple is on the depressions of the calyx and stem ends. If the total deposit is determined and the coverage is expressed as the average deposit per unit area, the result has little meaning. It is much higher than the deposit per unit area on the cheek surface and lower than the deposit in the ends. In table 1 are shown the results of coverage determinations of arsenical sprayed apples. There were 12 apples in each sample, and the cheek surfaces and the ends were analyzed separately. The arsenic in the stem and calyx ends varies from 24 to 42 percent of the total arsenic on the apples. A variation of this percentage renders the average deposit determined from the total deposit unreliable. The ends should be eliminated or analyzed separately.

The estimation of the area of the apples is the chief difficulty in determining coverage. Apples may be considered as spheres and their areas calculated from average diameters. This approximation may be sufficiently accurate for comparisons when the apples are similar in size and shape. A better approximation for certain varieties of apples, such as Winesap and Jonathan, can be made by calculating the area of the surface of revolution of a cardioid about its axis of

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symmetry. Barnes² computed the area of apples by the use of this geometric figure, using the equation

$$\text{Area in square inches} = 0.755 \left(\frac{1}{D} \right)^{2/3} W^{2/3}$$

where W is the weight of the apple in grams and D its density.

Perhaps a better method is as follows: The area of the surface of the revolution of the cardioid,

$$\rho = a(1 - \cos \theta)$$

is approximately $20.1 a^2$. a is determined from the apple measurements by multiplying the diameter perpendicular to the core by 0.385, and the diameter parallel to the core by 0.444 (fig. 1). Several Winesaps, picked in midsummer, were measured in this manner and their areas calculated; the peel was then removed in thin panels and the areas measured with a planimeter. The calculated and measured areas agreed within 3 percent. The stem and calyx ends may be removed with a cork borer and the areas removed deducted from the total area. The arsenic deposit may be removed from the apples with an alkaline-soap solution and acid rinse,³ the solution boiled down and digested with sulphuric and nitric acids,⁴ and the arsenic determined by the bromate method.⁵

TABLE 1.—Distribution of deposit on apples

Sample No.	Arsenic (As ₂ O ₃)				Sample No.	Arsenic (As ₂ O ₃)			
	Stem and calyx	Cheeks	Total	In ends		Stem and calyx	Cheeks	Total	In ends
	Milli-grams	Milli-grams	Milli-grams	Percent		Milli-grams	Milli-grams	Milli-grams	Percent
1.....	4.04	8.42	12.46	32.4	22.....	8.36	11.46	19.82	42.2
2.....	3.69	5.96	9.65	38.2	23.....	5.41	11.85	16.74	82.3
3.....	3.32	4.90	8.22	40.4	24.....	5.67	11.00	16.67	34.0
4.....	3.60	7.16	10.76	33.5	25.....	7.55	18.24	25.79	29.3
5.....	3.74	8.74	12.48	30.0	26.....	4.33	8.52	12.85	33.7
6.....	5.30	12.16	7.46	30.4	27.....	7.42	13.04	20.46	36.3
7.....	5.43	9.35	14.78	36.7	28.....	4.83	7.80	12.63	38.2
8.....	3.91	8.04	11.95	32.7	29.....	5.89	9.40	15.29	38.5
9.....	6.73	14.25	20.98	32.1	30.....	5.64	10.00	15.64	36.1
10.....	4.34	10.17	14.51	29.9	31.....	7.13	12.66	19.79	37.0
11.....	3.48	8.11	11.59	30.0	32.....	7.92	14.70	22.62	35.0
12.....	8.18	15.88	24.06	34.0	33.....	4.64	6.63	11.17	40.6
13.....	5.49	14.34	19.83	27.7	34.....	5.04	7.03	12.07	41.8
14.....	5.54	9.86	15.40	26.0	35.....	6.81	10.13	16.94	40.2
15.....	3.92	8.71	12.63	31.0	36.....	6.72	10.52	17.24	39.0
16.....	2.83	7.51	10.34	27.4	37.....	4.20	11.55	15.75	26.7
17.....	3.75	7.12	10.87	34.5	38.....	3.31	7.37	10.68	31.0
18.....	3.45	7.41	10.86	31.8	39.....	3.04	7.74	10.78	28.2
19.....	3.42	7.77	11.19	30.6	40.....	3.89	7.13	11.02	35.3
20.....	3.73	8.71	12.44	30.0	41.....	4.57	7.82	12.39	36.9
21.....	3.11	7.25	10.36	30.0	42.....	1.83	5.83	7.66	23.9

¹ BARNES, J. W. SAMPLING APPLES IN THE ORCHARD FOR THE DETERMINATION OF ARSENICAL SPRAY RESIDUE. A STATISTICAL STUDY. *Indus. and Engin. Chem.* 21: 172-174, illus. 1929.

² WICHMANN, H. J., MURRAY, C. W., HARRIS, M., CLIFFORD, P. A., LOUGHEEY, J. H., and VORHES, F. A., JR. METHODS FOR DETERMINATION OF LEAD IN FOODS. *Jour. Assoc. Off. Agr. Chem.* 17: 106-135, illus. 1934. See pp. 119-120.

⁴ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. . . . Compiled by the committee on editing methods of analysis. . . . Ed. 3, 593 pp., illus. Washington, D. C. 1930. See p. 307, no. 3.

⁵ JONES, W. C. REPORT ON BROMATE METHOD FOR THE DETERMINATION OF ARSENIC IN FOODS. *Jour. Assoc. Off. Agr. Chem.* 16: 75-77, 1933; 17: 202-204, 1934.

A simpler method of determining coverage consists in cutting a number of disks from the fruit with a cork borer and analyzing them. The area of a disk removed from a sphere may be calculated by the formula,

$$\text{Area of disk} = 2\pi R (R - \sqrt{R^2 - r^2})$$

where R is the radius of the apple and r is the radius of the disk. It is not necessary to calculate this area for each different apple radius; a table of areas may be calculated for each quarter- or half-centimeter radius interval and the areas of the disks taken from the nearest calculated radius. The disks are then digested⁴ and the arsenic

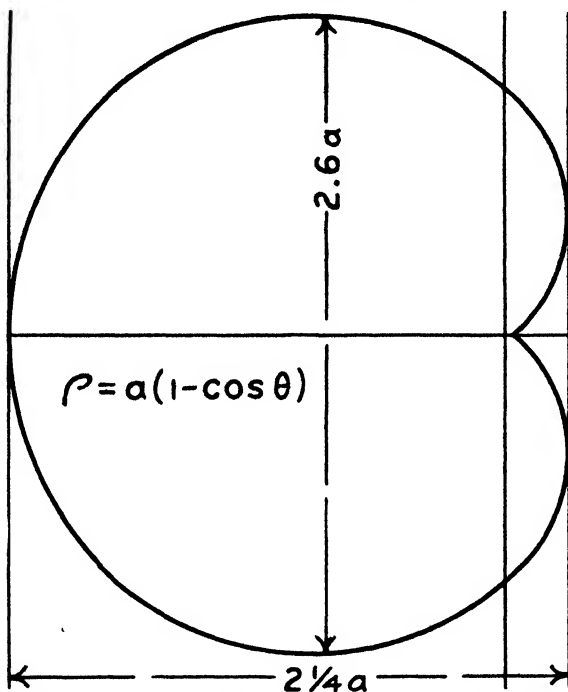


FIGURE 1.—Diagram showing the dimensions of a cardioid in terms of a .

determined by the bromate method.⁵ The digestion requires very little time.

In the work at this station samples of 12 apples were selected from each experimental plot and 6 or more disks were cut from each apple for analysis. A sample of this size proved satisfactorily reproducible. Barnes⁶ recommended samples of 50 apples each in order to obtain a result with a probable error of 5 percent. However, Barnes was interested in the deposit from a residue standpoint and the calyx and stem ends were analyzed with the cheek surfaces. The elimination of these ends, which is essential for accurate coverage information, reduces the variation considerably. It may be well to add that this source of variation is but one of several which occur between the application of spray and the eventual determination of coverage.

⁴ See footnote on page 1143.

⁵ See footnote on page 1140.

⁶ BARNES, J. W. See footnote 2.

For example, the type of spray mixture is of the utmost importance in effecting uniformity of spray application. Certain arsenical spray mixtures yield deposits which quickly reach a maximum as spraying is prolonged, whereas others recently developed at this station have the capacity of leaving deposits that increase almost indefinitely with prolonged spraying. Thus the careful and systematic application of spray materials so necessary in any case becomes doubly important. Should one tree be sprayed, let us say, twice as heavily as its neighbor the amounts of the arsenical spray deposits on the two trees might readily vary 100 percent from this source alone.

Apples selected for analysis must be handled carefully and the surfaces to be analyzed should not be touched. The apples should be held by the stem and calyx ends. While being transported to the laboratory they may be impaled on nails driven through boards. A cork borer with an inside diameter of about 1.8 cm is convenient for disk cutting.

According to the writers' experience, the Gutzeit method, though useful for determining minute amounts of arsenic, such as occur in residue samples, is less suitable than the bromate method for ascertaining the relatively large amounts which are encountered in measuring deposits. In the latter case the 10 percent error expected in the Gutzeit method⁷ compares to less than 1 percent in the bromate method.⁸ Coverage determinations by the disk method correlated unusually well with control on arsenical sprayed plots.

⁷ BARNES, J. W., and MURRAY, C. W. ACCURACY OF THE GUTZEIT METHOD FOR THE DETERMINATION OF MINUTE QUANTITIES OF ARSENIC. *Indus. and Engin. Chem. Analyt.* Ed. 2: 29-30, illus. 1930.

⁸ JONES, W. C. REPORT ON ARSENIC. *Jour. Asso. Off. Agr. Chem.* 16: 325-329. 1933.

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